



















JOURNAL  
OF  
MORPHOLOGY

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VOLUME 21  
1910

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY  
PHILADELPHIA

E687(24)

1193

COMPOSED AND PRINTED AT THE  
WAVERLY PRESS  
By THE WILLIAMS & WILKINS COMPANY  
BALTIMORE, U. S. A.



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1910

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# DIVERSITY IN THE SCUTES OF CHELONIA.\*

ROBERT E. COKER.

WITH TEXT FIGS. A TO Q AND PLATES I TO XIV.

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\*This paper was completed in June, 1906. While unavoidable circumstances have prevented its earlier appearance, the text has had no material alteration.

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### INTRODUCTION.

At the beginning of an inquiry regarding the diamond-back terrapin, *Malaclemmys centrata* (Latr.), undertaken in 1902, my attention was attracted by the frequent instances of striking deviation from the "normal" number and arrangement of the horny scutes of the carapace. The anomalies seemed of sufficient interest to justify a record of the facts, and accordingly were included in the notes made in connection with the economic study then in progress. Later, the study was extended in some lines, as it was seen that the anomalies might have some significance for theoretical interpretation or experimental study.

For kind permission to use in this paper such data as was obtained while employed in the economic study of the terrapin, my acknowledgment is due to Hon. George M. Bowers, United States Commissioner of Fish and Fisheries, and to Professor J. A. Holmes, State Geologist of North Carolina. My thanks are also due to the officials of the Bureau of Fisheries and to Dr. Caswell Grave, Director of the Fisheries Laboratory, for many courtesies extended to me while occupying a research table in the Laboratory.

I wish to make grateful acknowledgment of my indebtedness to Prof. W. K. Brooks, under whose guidance the study has been prosecuted.

#### *Character of the Diversity.*

At first I was particularly impressed by the comparatively great prevalence of apparent "abnormalities" in the number of horny

scutes of the carapace. Longer acquaintance with a number of individuals of this species that were kept under observation at the Fisheries Laboratory at Beaufort, North Carolina, and with the eggs, embryos, and young of the loggerhead sea-turtle *Thalassochelys caretta*, made apparent a wide diversity in many respects. Among the characters in which the turtles and eggs manifested striking individual differences, we may mention shape and size of eggs and of young, shape and size of head, shape of carapace, depth of body, color pattern, boldness, habits of feeding and of hibernating and of moulting, rate of growth, etc. It seemed that a diversity in respect of scutes and plates was no more than one would expect in view of the diversity shown in so many other respects. The aphorism that no two individuals are exactly alike would seem to apply with pre-eminent fitness to certain species of turtle.

Nevertheless, there are certain features of these "abnormalities" which the observer cannot but be impressed with, and which seem to suggest some special significance. These features are:

1. *The frequent recurrence of certain more or less regular scutes in definite positions.*

2. *The striking correspondence of recurring abnormal elements of one species to those of another, and sometimes to normal scutes of other species.* Thus—

- (a) In 31 specimens of new-born green turtles\* 44 different abnormalities were noted, but these were reducible to 11 types; or, to omit variations which occurred not more than twice and are possibly *coincidences*, we have 39 abnormalities of 7 types. The most anterior scute of the plastron, the normally unpaired inter-gular, was in 6 specimens represented by a pair of scutes, and in 9 other specimens was partially divided. This scute occurs in few genera, but in the normal conditions of *Macrolemmys* and of *Chelys* (Gadow, '01, p. 325), there is found in this position a pair of scutes.

- (b) In 3 specimens of the same lot a rectangular scute appeared in the neural series between the normal fourth and fifth shields. An almost exactly similar scute occurs twice as the only abnormality of dorsal scutes noted in 4 specimens of *Thalassochelys* (*Colpochelys*)

\*See author's previous paper, '05a, p. 23.

*kempii* Garman; it is noted in several specimens of *T. caretta*, and in other species; and a practically identical scute is figured by Boulenger for the remote species *Chelodina novæ-guinææ* Boulenger (Boulenger, '89, Pl. 5), but the author does not state whether or not the presence of this scute is normal. There seems some ground for the belief that such a definite recurrence of a scute of fairly regular shape and position has some special significance.

(c) In 3 of the above 31 turtles, the nuchal was represented by a pair of scutes. The same abnormality occurred in 10 of 243 specimens of *Malaclemmys* (Tables I-IV) and in 9 others the nuchal, though unpaired, was marked by a median longitudinal groove. This shield occurs in paired condition in several specimens of *Thalassochelys*. In some species, notably in *Chrysemys guttatus*, the nuchal often shows a distinct notch in the anterior margin.

(d) In most genera of land and fresh-water turtles, *axillary* and *inguinal* scutes are found anterior and posterior, respectively, to the bridge and just beneath the marginals (Fig. B). These scutes are regarded as the remnants of an ancestral series of *inframarginals* separating the pectoral, abdominal, and femoral scutes of the plastron from the marginals. *Malaclemmys centrata* has "normally" only the axillary, yet over 21 per cent, of 244 specimens examined, possess inguinals, of varying size, on one or both sides.

A number of other cases might be mentioned of recurring scutes in definite positions in the neural, costal, and marginal series, but these instances are sufficient to illustrate the nature of the conditions that have led some recent writers to assume that these abnormal scutes are *atavisms* and that, as such, they have a comparative value, similar to that of normal scutes, but of much more significance, since they may point more directly to remote ancestral forms.

Finally, it must be said that many other scutes are noted which are not of these definite types, but which are perhaps not less significant.

In the present paper I will present my observations on the scutes of two species of turtles, and then, in the light of these and other observations, will inquire into the basis for an atavistic interpretation. It is necessary first to have in mind the phylogenetic sig-

nificance attached to normal scutes. Some representative views are given in the next section.

*Some Recent Views regarding the Phylogenetic Significance of Normal and Abnormal Scutes.*

O. P. Hay's view as to the origin of the carapace (Hay, '98) is quite important in this connection, for he seems to have been the first to realize the probable phylogenetic significance of the epidermal scutes. Previous discussions had centered chiefly about the dermal and periosteal bony skeleton.

Hay distinguishes three kinds of bone in the carapace: (1) cartilage bone; (2) true dermal bone, developed in the skin itself, and comparable to the osteodermal plates of *Dermochelys* and of the crocodiles; (3) fascia bone, originating subcutaneously by ossification of the fascia below the skin. The present carapace owes its phylogenetic origin to the complete fusion of cartilage and fascia bone. True dermal bone has probably completely or almost completely disappeared from the carapace of modern Thecophora. But, according to Hay, the ancestors of all turtles, *Thecophora*, as well as *Athechæ*, have possessed an armor of true dermal bony plates, probably mosaically arranged, and with twelve well differentiated keels. Of the keels, five were dorsal (one median and two pairs lateral), two were marginal, and five ventral (one median and two pairs lateral). The plates of this armor were adapted to previously formed epidermal scutes of the same mosaic pattern. This osteodermal armor is retained in *Dermochelys* with essentially the primitive pattern, but the epidermal shields are lost in the adult and indicated only in the young.

In Thecophorous turtles the mosaic 12-keeled dermal armor underwent important modifications partly in correlation with the greater development of the internal skeleton. The plates became very much reduced in number, a few of the plates of the keels, growing in size, and assuming the whole function of the protective armor, crowded out many of the keel plates, and all of the smaller plates between the keels. Some of the keels too were lost. As the result, we may suppose a dermal carapace of twelve series of scute-covered plates, with



a comparatively small number of units in each series. This armor overlapped, or "broke joints," with the yet imperfectly developed cartilaginous carapace, but with the further development of the latter, and the consequent loss of the usefulness of the outer armor to the animal, the dermal skeleton became reduced and finally disappeared. The epidermal scutes, corresponding to these plates, remain, however, and from their present arrangement in the carapace of Thecophorous turtles we are enabled to infer something of the modifications which the dermal armor underwent. Probably the last remnants of the dermal plates persisted as small ossicles at the keel prominences that are noticeable in adults of some species, but especially in the young of certain species (e. g., of *Thalassochelys*, cf. Fig. 74). In the further course of evolution these remnants became either completely reduced or merged into the deeper plates underlying them; but Hay has observed at least one, and probably two such ossicles in the neural series of a fossil specimen of *Toxochelys*.

Thus, in Hay's view, the series of scutes of the carapace are directly homologous to the series of epidermal areas overlying the plates of the keels of a young *Dermochelys*. The neural series corresponds to the median dorsal keel. Of the two lateral dorsal keels, one is represented by the costals, while the other is lost in most turtles, but preserved as a short series of supra-marginals in *Macroclommys*. Marginal keels correspond to marginal scutes. Of the two lateral ventral keels, the internal gave rise to the plastral scutes, while the external is more or less reduced, but still survives in most turtles, either as a continuous series of inframarginals (sea turtles, etc.), or as isolated axillary and inguinal shields. In some land tortoises this series is entirely unrepresented. The median ventral is almost entirely lost, but remnants are seen, for example, in the characteristic unpaired intergular of *Chelodina*, and in the occasional occurrence in other species of small unpaired scutes in the median line of the plastron. Such a scute is most commonly found just at the apices of the gulars and is referred to as an "intergular," or, better, as an "*interplastral*."

An hypothesis advocated by Newmann follows in logical order

upon Hay's view; but a view advanced by Gadow should be referred to here, as it is not only the next in chronological sequence, but is the first in which the atavistic interpretation was applied in a comprehensive way to the abnormalities of scutes.

The two striking features of Gadow's paper are: (1) the attempt to classify the variations and interpret them as *reversions to ancestral conditions*; and (2) the hypothetical explanation of these atavisms as stages in ontogeny or *arrests of development*. In his own concise words, the abnormalities are viewed as "simply ontogenetic stages, passing reminiscences of earlier phylogenetic conditions" (Gadow, '05, p. 638).

Originally, according to this author, there was a scute for each dorsal plate. Thus, as there are eight transverse series of dorsal plates, each series consisting of a median neural and a pair of lateral costals, so there were originally at least eight transverse series of dorsal scutes, scute and plate coinciding. But a process of reduction ensued. First, by the reduction of a pair of costals (probably the second), the scutes of neural and costal series came to dovetail into one another, and this dovetailing plan was subsequently retained throughout all stages. Gradually the number of scutes was reduced by the suppression—first, of the second costals, then of a neural; then fifth costals, fifth neural, and finally by the fusion of the last two costals; thus was attained the present typical condition of *Thalassochelys* with six neurals (including the nuchal), and five pairs of costals. When turtles are found with more than this number of scutes the condition is to be regarded as "reminiscent" of one of these phylogenetic stages. The order of suppression of scutes given above is inferred from the relative frequency of recurrence of "supernumerary" scutes in the several positions. Thus far, Gadow's interpretation, though open to criticism, is interesting and suggestive. When he goes further, and regards these atavisms, when found in adults, as instances of arrested development, or, when found in younger turtles, as proper stages in ontogenetic recapitulation of the phylogenetic stages, his position seems untenable on the basis of any facts now in hand, as the writer has previously shown ('05 a), and as Newmann's observations also indicate (Newmann, '06, p. 92).

Newmann ('06), who has made the most extended study of the abnormalities of scutes and plates, lays much stress on the phylogenetic significance of scutes, but departs materially from Hay in that he regards *Dermochelys* as out of the line of descent of Thecophorous turtles, as "an abnormal and perhaps highly specialized form." This is a position which Baur held and zealously defended in a series of papers from 1886 to 1896 ('86, '88 a and b, '89 a and b, '90, '96).<sup>1</sup> The view was adopted by Case ('97).

Newmann bases his view primarily on a study of the assumed atavisms and of the color patterns of the carapace, but in part also on the comparison of the different scute-plans normal for different species of turtles. The keynote of his paper is expressed in the words: "Careful study has convinced me that these abnormalities are to be considered not as meaningless anomalies but as examples of systematic atavism in the sense of de Vries. From this standpoint it seems possible to throw some light on the phylogeny of *Chelonia*."

These abnormalities as Newmann regards them are reversions, not to the keel plan of *Dermochelys*-like turtles, but to a plan essentially similar to that found in the "tail-trunk" of modern *Chelydra*, where he believes the primitive condition of the scutes is most nearly preserved. There he finds seven principal rows of scutes and, alternating with them, seven subordinate rows of smaller or less regular scutes. To homologize these with the series of scutes in the carapace and plastron,—the seven principal rows correspond to neural (one), costal (two), marginal (two), and plastral (two); these rows are found in all carapaces (except those of the Trionychoidea). The seven subordinate rows correspond to (a) paired neuro-costals (lost in all turtles); (b) paired supramarginals (preserved normally only

<sup>1</sup>Newmann's statement ('06, p. 99) that Baur with Hay regarded *Dermochelys* as the ancestral form, seems based on a preliminary note by Baur dated October 6, 1886, and appearing in the *American Naturalist* for January, 1887. Subsequent to the writing of this preliminary note, even prior to its appearance, Baur had discarded the old and generally accepted view, so that his complete paper, dated October 26, and appearing in the *Zoologische Anzeiger* for November, 1886, announced unmistakably the view which he thereafter maintained.

in *Macrocllemmys*); (c) paired inframarginals (preserved in sea turtles and, as inguinals and axillaries, in most other turtles, but entirely lost in a few land tortoises); and finally, (d) unpaired interplastrals—preserved normally only as a single intergular in a few species, as *Chelodina*. In general, the principal rows are retained in all turtles, but the subordinate rows are largely lost. Remnants of these latter series, however, are retained in the normal condition of some species, and, further, *reappear as atavistic abnormalities* in individuals of species that do not normally possess them. Thus, *inframarginals* were found “abnormally” in *Graptemys geographica* and *Chrysemys marginata*, etc.; *interplastrals*, in specimens of *Chrysemys*, *Chelydra*, and *Graptemys*. Newmann does not seem to have found either *neuro-costals* or *supramarginals* as individual variations.

Furthermore, Newmann believes that the number of scutes of the neural and costal series was formerly about twice as great as at present, and that alternate scutes have been forced out and lost, but that these, again, recur in individuals as atavisms.

There has been, then, a reduction both in the number of rows and in the number of scutes in a row; but atavistic occurrences of the lost elements are met with in abnormal individuals of many species. From a systematic study of these atavisms, we may infer something of the evolutionary history of the Chelonian carapace and plastron.

Newmann differs from Hay in supposing fourteen original rows as opposed to Hay's twelve; in seeking the ancestral type not in *Dermochelys*, but in the disposition of the scutes of the base of the tail of *Chelydra*; in supposing but a comparatively small number of original scutes in each series of the carapace; and in seeking a basis for his views in the systematic study of atavisms.

The question of the value of the anomalous scutes as evidence of phylogeny will be discussed in a later portion of this paper.

#### *Use of Terms.*

The term “scute” will apply invariably to a horny shield of the epidermal carapace; “plate” to an element of the bony carapace; “seam” refers to the line of separation of adjacent scutes, as “suture” to that of adjacent bones.

The nomenclature of the scutes will be clear from Text-figs. A, B and C.

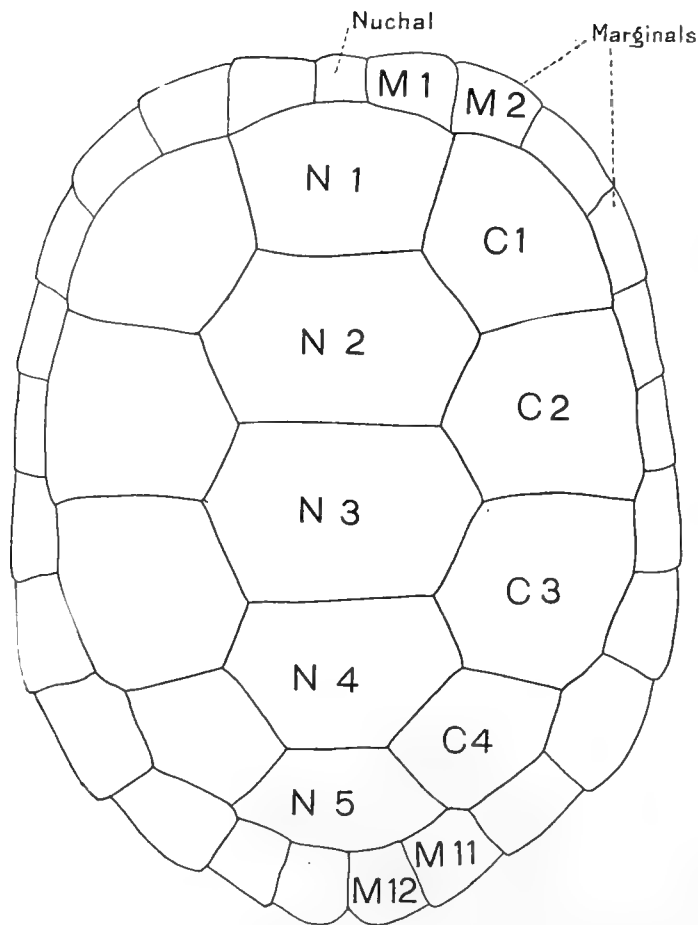


FIG. A. Normal carapace of *Malaclemmys centrata* (Latr.) (No. 253 of Table V). A median series of unpaired scutes composed of a small anterior *nuchal*, followed by 5 large *neurals*; 4 pairs of *costals*; 12 pairs of *marginals*.

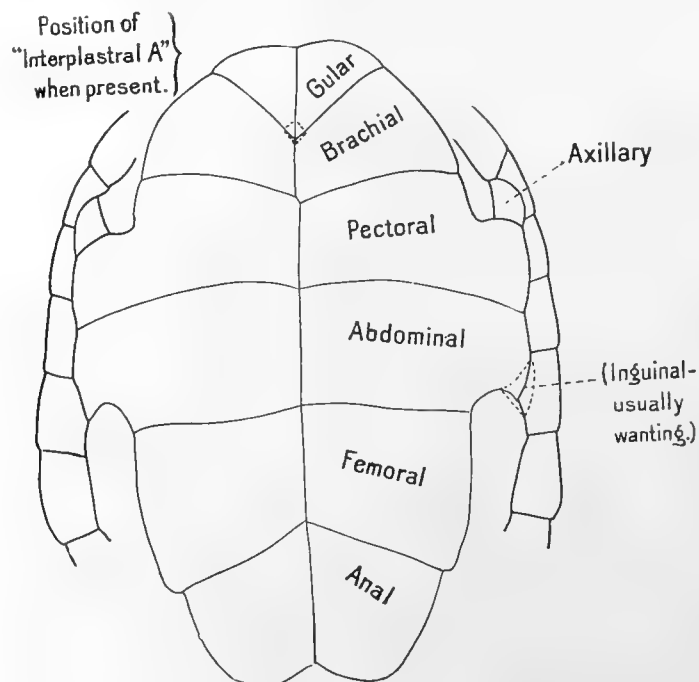


FIG. B. Normal plastron of *Malaclemmys centrata* (Latr.). Dotted lines indicate the positions of *inguinals* and *interplastrals*, normally absent.



(See also "abbreviations," below.) The *nuchal* is not, for present purposes, included in the *neural* series. As the term "intergular" is commonly applied both to the unpaired scutes, anterior to the gulars (typical of certain species) and also to a smaller or larger median scute at the posterior-median angles of the gulars (of other species), I will use the name *intergular* only in the former sense, for a median scute on the anterior margin between the gulars (Fig. C); *interplastral* is applied to other median shields of the plastron (Fig. B). The terms "inframarginal" and "submarginal" must be distinguished. *Inframarginal* applies to a series of scutes separating pectoral and abdominal shields from the marginal series (a typical series in *Cheloniidae*, etc. (Fig. C), and, presumably, represented in other species by the *axillaries* and *inguinals*) (Fig. B); *submarginal* is used, as applied by Baur ('90), to anomalous scutes found between the inframarginals and marginals (Fig. C).

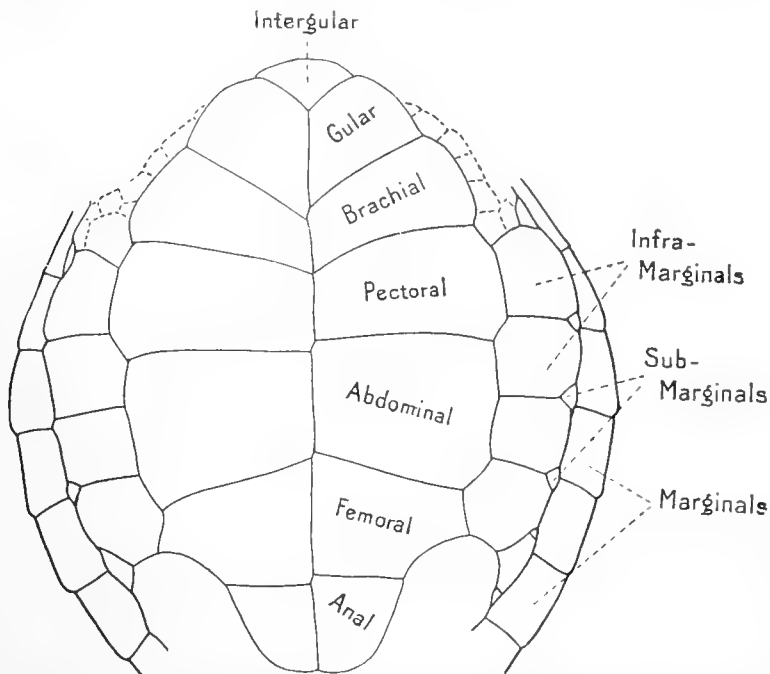


FIG. C. Plastron of a specimen of *Thalassochelys caretta* (L.). Note the anterior unpaired *intergular*, the series of *inframarginals*, and the small anomalous *submarginals*.

It seems impossible to escape the use of words that could be taken to imply more than is intended. It may, for instance, be perfectly *normal* for one turtle to have a scute not possessed by most of its fellows of the same species, but, for our present purpose, the number and arrangement typical of the species is termed "normal." A scute not found in the typical plan will be described as an "abnormality," an "anomaly," a "supernumerary" scute, or a "variation." The collective term least liable to mislead is "diversity," and this is used whenever practicable.

*Explanation of Tables.*

*Abbreviations:* "L," left; "R," right; "N," neural; "C," costal; "M," marginal. Thus "N1" denotes the first (most anterior) neural, LC4, the fourth (most posterior, typically) left costal, etc.

In the "inguinal column," "L" denotes the presence of a left inguinal, "R" of a right, "L R" of both. If the left inguinal is distinctly larger than the right, this is indicated thus "L > R," or, if the reverse is the case, "L < R."

In the "Remarks" column "Supn'y" is used for *supernumerary*.

In the "nuchal" column, "pr" indicates that the nuchal is represented by a pair of scutes, "f," that it is marked by a longitudinal furrow, not distinguishable as a seam.

The letter "x" in any column refers the reader to the "Remarks" column.

In the "figures" column, letters refer to text figures, numbers to the figures on the plates.

Regarding the identification of scutes, it is not always clear to which series in the carapace an anomalous scute should be referred, or in fact whether there is reason in ascribing it to any normal series. But for convenience of description scutes will, when possible, be referred to normal series.

In dealing with forms of so high a market value as the diamond-back terrapin (Tables I to V) it was not always possible to retain the specimens for further study. But in regard to scutes alone, revision of judgment is necessary in a comparatively small number of cases, and a field sketch frequently helps to remove this difficulty.

Regarding the figures, some are photographs, some tracings from photographs, and some camera sketches, while others are field sketches (see *Explanation of Plates*," etc.). No greater accuracy is claimed for the field sketches than that they represent, somewhat diagrammatically, the position, shape and proportionate size of the scutes depicted.

As diversity manifests itself in so many respects, we must of necessity disregard most individual differences, and consider only certain defined "abnormalities." In the tables that follow, a turtle is "normal," unless it possesses *more or less than the number of scutes* typical of the species, shows instances of *partial division* or *partial fusion* of scutes, has a median plate marked with a *distinct and complete median groove*, exhibits such asymmetry as with *one scute of a pair twice as large as its mate*, or has scutes not firmly united to the bone.

TABLE I.

*Malaclemmys centrata* (Latr.), Beaufort, N. C., 1902.

Serial Number.	Length of Plastron in inches.	Sex.	Rings of Growth.	SCUTES.							REMARKS.	Figures.
				Nuchal.	Neural.	Costals.		Marginals.		Inguinals.		
						Left.	Right.	Left.	Right.			
1	2.6		2	1	5	4	4	12	12		Normal.	20
2	2.7		2								Normal.	
3	2.7		2	f								
4	2.7		2		5	5	4	11	11	L R	M11 broad, evidently representing normal 11 and 12; minute scute anterior to LC1.	
5	2.8										Normal.	{ 3 4
6												
7			2							R	R. inguinal small.	
8		2.9		2					11	11		
9	3	+	3			4	5			L	Supn'y costal small, anterior, L axillary $\frac{1}{2}$ R in size.	
10	3		2								Normal.	
11												
12			2								Normal.	
15		3.1		2							Normal.	
16	3.2		2								Normal.	
17	3.5		4								Normal.	
18	3.5	O <sub>3</sub>									Normal.	
19		O <sub>3</sub>									Normal.	
20		O <sub>3</sub>									Normal.	
23		3.6	O <sub>3</sub>									
24	3.6	O <sub>3</sub>								L R		
25		O <sub>3</sub>										
26		3.7	O <sub>3</sub>								Normal.	
27		3.7	O <sub>3</sub>							L R		
28	3.8	O <sub>3</sub>	5								Normal. No. 29 with 8 rings; Nos. 30 and 34 with 5.	
29		O <sub>3</sub>	x									
34		O <sub>3</sub>										
35		3.8	O <sub>3</sub>						13	13		
37	3.8	O <sub>3</sub>						13	13		Pair of scutes between N5, C's 4 and M's 12 and 13 (= 5th pr. of costals, or 6th neural divided?) Supn'y marginal posterior.	
38		O <sub>3</sub>		x				13	13			
39	3.8	+								L R	Normal.	
40	3.8										Normal.	
41	3.9	O <sub>3</sub>									Deep notch between last pair of marginals.	
43		O <sub>3</sub>		pr							Deep notch between RM10 and 11 (Scar?).	
44		O <sub>3</sub>									L. axillary wanting, RM11 and RM12 continuous distally, divided proximally.	
45	3.9	O <sub>3</sub>								L R	Normal.	
46	3.9	O <sub>3</sub>									Normal, except that 2d, 3d, and 4th pairs of plastral scutes overlap, respectively, the scutes immediately posterior.	
47	3.9	O <sub>3</sub>							x	R	Normal. (No. 53 with 4 rings of growth. Seam between N4 and N5 oblique.)	
48	3.9										Normal.	
49											Minute 5th R C. about 1-40 size of 4th. Seam between N4 and N5 oblique.	
50		3.9										
51	4.	O <sub>3</sub>			x							
54												
55		+										
56		+			x	4	5					
57	4.	O <sub>3</sub>	4							L < R		
58	4.	O <sub>3</sub>								L R		
59	4.	O <sub>3</sub>									L. axillary wanting.	
60	4.1	+									Normal.	
61												
62		4.1	O <sub>3</sub>	3						L R	LC4 reduced.	
63	4.1	O <sub>3</sub>				5	4					

(Continued on next page.)

(Continued from preceding page.)

SCUTES.															Figures.
Serial Number.	Length of Plastron in inches.	Sex.	Rings of Growth.	Nuchal.	Neural.	Costals.		Marginals.		Inguinals.	REMARKS.				
						Left.	Right.	Left.	Right.						
64 } 66 } 67 }	4.2	{ { {									Normal.	1			
69 }	4.3	{									Normal. No. 68 with 4 rings.				
70 }	4.4	{ +O+													

TABLE II.

*Malaclemmys centrata* (Latr.), Beaufort, N. C., 1903.  
(A few from Brunswick County, N. C.)

A.—MALES.

Serial Number.	Length of Plastron in inches.	SCUTES.							Figures.	
		Nuchal.	Neurals.	Costals.		Marginals.		Inguinals.		REMARKS.
				Left.	Right.	Left.	Right.			
93	3.	pr				13	13		Normal.	
94	3.3								Normal.	
95	3.6								R axillary $\frac{1}{2}$ of L in size.	
96	3.6								Axillaries wanting.	
97	3.6								Normal.	
98	3.6								Normal.	
104	3.7								M13 small.	
105	3.7								Normal.	
107	3.8								Axillaries not fixed to bone beneath,	
108	3.8								but movable; L<R.	
109	3.8	f						R	Normal.	
113	3.8								Normal.	
114	3.8									
115	3.8									
116	3.8									
117	3.9	pr						L R	R axillary twice as large as L.	
120	3.9								Normal.	
121	3.9									
122	3.9									
123	4.									
124	4.2									
125	4.3								f	

B.—FEMALES.

126	3.				13	13		2 marginals in place of normal M1.	13
127	3.2						R	Normal.	
128	3.3				12	13		2 scutes in place of normal RM12.	
129	3.6			5 4				Small LC5.	
130	3.6			5 4	12	13		2 scutes in place of normal RM1.	9
131	3.7							Small L costal anterior to normal first.	
132	3.9							Supn'y plastral scute between femoral and anal of R side.	K
133	3.9			5 4				Small 5th. LC.	10
134	3.9							Normal.	
135	3.9							R axillary twice L in size.	
136 }	4.							Normal.	
137 }	4.								
138 }	4.						L	L inguinal small.	
139 }	4.	x						Small scute between N1 and 2, similar in size and shape to supn'y scute of No. 150, but entirely to left of median line and without keel. (Cf. fig. 6 of No. 150.)	
140 }	4.						R		
141 }	4.1						L R		
142 }	4.2				x	x		LM11 is less than $\frac{1}{2}$ LM10 in size; RM11 is less than $\frac{2}{3}$ RM10.	
143 }	4.2						R	Normal.	
144 }	4.2								

(Continued on next page.)



(Continued from preceding page.)

Serial Number.	Length of Plastron in inches.	SCUTES.							Figures.
		Nuchal.	Neurals.	Cos-tals.	Marginals.		Inguinals.	REMARKS.	
				Left. Right.	Left.	Right.			
145	4.3					13		Supn'y M posterior to LM3, barely showing dorsally. L abdominal and R femoral meeting for more than $\frac{1}{4}$ mesial border of femoral.	37 38
146 } 148 } 149	4.4 4.4		x	?			L < R	Normal. 2 scutes occupying place of N5. Ing.	
150	4.5		x					Small scute anterior to L side of N4, extending barely over median line and bearing keel prominence.	6
151	4.5		x	4 5	12 13			Region of N3, N4 and N5 occupied by 6 scutes, the last incompletely divided. L axillary wanting, R small.	7 15
152	4.5		x				L R	Seam between N4 and N5 distorted slightly.	
153	4.5				x x			Normal, except that LM12 and RM-12 were very narrow proximally ( $\frac{1}{2}$ width of 11) but of usual width distally.	
154 } 156 } 157 } 158 } 159 } 160 }	4.5 4.6 4.6 4.6 4.6						L R	Normal. L axillary wanting.	
161	4.7		x				L R	Normal. Inguinals small. Seam between N4 and N5 distorted.	E
162	4.7		x		13 13			Seam between N4 and N5 oblique.	
163	4.8						L	Normal, but axillaries small.	
164	4.8							L inguinal small.	
165 } 166 }	4.8							Normal.	
167			x	5 4				N3 incompletely divided, N4 represented by 2 scutes.	16
168	5.3	pr	x				L R	2 scutes in place of N5.	
169	5.3						L R	Normal.	
170	5.4							Normal.	
171	5.5							Normal.	
172	5.5								
173	5.6						R		
174	5.9				x x		L R	12th margi'ls asymmetrically placed, LM12 having a position in median line.	26
175	6.4						R		
176	6.8				x			RM's 2 and 3 fused proximally.	34

TABLE III.

*Malaclemmys centrata* (Latr.). Observed at Crisfield, Md., 1903.

## ALL FEMALES.

Serial Number.	Length of Plastron in inches.	SCUTES.							Figures.	
		Nuchal.	Neurals.	Costals.		Marginals.		Inguinals.		REMARKS.
				Left.	Right.	Left.	Right.			
177	4.									
178	4.3					13	13		Normal. LM12 and RM12 each represented by 2 scutes.	30
179 }	4.3								Normal.	
180 }	4.3								2 scutes in region of N5, one quite minute. Small interplastral.	{ 47 H
181	4.3		x	5	4					
182	4.3	f						R		
183	4.4					12	13		RM12 and 13 equivalent to LM12.	33
184	4.4								Normal.	
185	4.5	f				x	x		12th marginal small, unequal.	29
186 }	4.5								Normal.	
187 }	4.5									
188 }	4.6								Normal.	
190 }	4.6								Normal, except for slight distortion of posterior scutes of carapace.	28
191	4.6		x						Normal.	
192 }	4.8								Normal.	
194 }	4.8								Region of N3 to N5 occupied by 5 scutes.	8
195			x	5				L R		
196	4.8	pr	x	5		13	13		Region of N5 occupied by 2 scutes.	{ 31 42
197	5.1							L R	Inguinals small.	
198	5.4		x	?	?	13	13		3 scutes between N5 and marginals.	45
199	5.4		x	5				L R	Region of N5 occupied by 3 scutes.	43
200	5.4	pr								35
201 }	5.5								Normal.	
206 }	5.5									
207	5.5							L R		
208	5.5	f						L R	Furrow a little to right of median line of nuchal.	
209	5.5					13	13	L R	13th marginals small, with deep notch in R. Broad notch in outline of carapace in region of RM11.	32
210	5.5	pr	x	?	5		x		Very abnormal carapace: see photograph.	{ 5 17
211	5.6	f	x						Median furrow on nuchal and on anterior halves of N1 and 2.	
212	5.6								Normal.	
213	5.6							R		
214	5.8								Normal.	
215	5.8							L R		
216	5.9								Normal.	
217	6.		x		x		13		2 scutes posterior to N5. RC5?	46
218	6.3		6	6	6			L		{ 41 44
219	6.4							L < R	L inguinal small.	
220	6.4							L	Normal.	
221	6.4								N5 represented by 2 scutes.	48
222	6.4		x						N4 elongated antero-posteriorly; N5 reduced, pair of minute triangular sup-	
223	6.5		x						n'y scutes.	
224	6.6								Normal.	
225 }	6.8								Normal.	
226 }	6.8									

Normal.  
LM12 and RM12 each represented by 2 scutes. 30

Normal.  
2 scutes in region of N5, one quite minute. Small interplastral. { 47  
H

RM12 and 13 equivalent to LM12. 33

Normal.  
12th marginal small, unequal. 29

Normal.

Normal, except for slight distortion of posterior scutes of carapace. 28

Normal.  
Region of N3 to N5 occupied by 5 scutes. 8

Region of N5 occupied by 2 scutes. { 31  
42

Inguinals small.  
3 scutes between N5 and marginals. 45

Region of N5 occupied by 3 scutes. 43  
35

Normal.

Furrow a little to right of median line of nuchal.  
13th marginals small, with deep notch in R. Broad notch in outline of carapace in region of RM11. 32

Very abnormal carapace: see photograph.  
Median furrow on nuchal and on anterior halves of N1 and 2. { 5  
17

Normal.

Normal.

Normal.  
2 scutes posterior to N5. RC5? 46

41  
44

L inguinal small.  
Normal.  
N5 represented by 2 scutes.  
N4 elongated antero-posteriorly; N5 reduced, pair of minute triangular sup-n'y scutes. 48

Normal.

Normal.

TABLE IV.

EMBRYOS AND NEWBORN—*Malaclemmys centrata* (Latr.).

Serial Number.	Length of Plastron in Inches.	Length of Carapace in Inches.	SCUTES.						Inguinals.	REMARKS.	Figures.
			Nuchal.	Neurals.	Cos- tals.		Margi- nals.				
					Left.	Right.	Left.	Right.			
227	.32	.52	f								
228	.35	.51	pr	x			13	13		3 scutes in the place of N5. <i>Normal.</i>	22
229	.40	.58									
230	.45	.65									
231	.62	.77	x						L R L>R	Inguinals small; slight notch in nu- chal anteriorly. <i>Normal.</i>	
232	.71	.92									
233	.78	.76		x	4	5	12	12		Very abnormal in shape but scutes adhere to typical series arrangement; neural scutes most abnormal (v. fig.25). Supn'y scute in L series of plastron pos- teriorly. L axillary wanting. A R in- framarginal? <i>Normal.</i>	{ 25 L
234	.78	.99									
235	.80		x	x	x		13			Misshapen: nuchal pushed over to R side; 12th L marginal represented by 2 scutes, LC4 reduced, N6 wider on L side. Posterior part of plastron short- ened and distorted; large umbilical scar. (About 7 months old. Hatched from eggs laid in confinement.) <i>Normal.</i>	
236	.90	1.00									
237	.95	1.11			4	5					
238	.96	1.14									
239	.96	1.15									
240	.98	1.08							R	About 7 months old. Hatched in pound at Crisfield, Md. <i>Normal.</i>	
241	.99	1.16									
242	1.10	1.24								<i>Normal.</i> (Cf. note on No. 240.)	
243	1.11	1.23							L R	(Cf. note on No. 240.)	

TABLE V.

SELECTED ABNORMAL TERRAPIN—*Malaclemmys centrata* (Latr.).

Serial Number.	Length of Plastron in inches.	SCUTES.								Figures.	
		Nuchal.		Neurals.		Costals.		Marginals.			REMARKS.
						Left.	Right.	Left.	Right.		
244		pr	x	5	5					Median furrow on anterior portions of N1 and N2; supn'y costals anterior.	2
245	(3.)	pr		5	5	11				Supn'y costals anterior; LM11 nearly equivalent to RM11 and 12. (Carapace distorted in drying, and broken at "x".)	18
246	3.5		x	5	5					Supn'y costals anterior and small; seam between N4 and 5 distorted slightly.	19
247	3.4		x							N5 represented by a large and a small scute. (Note that, with seam removed there would be a scute of nearly normal size, and that the areolæ together would make a normal areola.)	24
248	3.6					x				One medium, one small, and one minute scute occupying place of LM9—(Wound?).	40
249	3.6	pr	x	x	x	x	x			Asymmetry of scutes and distortion of seams in posterior region of carapace (Neurals, Costals and Marginals).	36
250	3.6			6	6	4	12	13		v. fig. 50, also description p. 26.	50
251	3.6		x	5	4					7 scutes in neural series (2 in place of 4th).	14
252	4.		x					x		5th neural represented by 2 scutes of unequal size; RM12 incompletely divided.	49
253	4.3									Small L inguinal.	F
254	4.3									Large inguinals; small scute immediately anterior to L inguinal.	G
255	4.6	x		5	5	13	12	x		Of abnormal shape; 12th L marginal represented by 2 scutes; only 12 R marginals, but between RM11 and RM12, a small gap occupied by soft skin. Supn'y costals posterior; also a very small scute between LC4, LC5 and marginals. Nuchal partly divided; inguinals. (Vertebral column distorted to left posteriorly. L side of carapace flattened, somewhat concave posteriorly. Shell suggestive of injuries rec'd in embryonic stage. Evidence of much shedding, horny covering thin and smooth.)	39
256	4.6		x	?	?	13	12			RM12 equivalent to LM12 and 13. 4 scutes occupying a space almost exactly coinciding with that of normal N5. Small inguinal.	23
257				5	4					LC3 followed by two scutes, which together are almost exactly equivalent to a normal C4 (cf. RC4).	27

## PART I. MALACLEMMYS.

The observations are presented as made on individual specimens in the tables of Section 1, and are treated in classified form in Section 2. The classification of abnormal neurals and costals leads to the discussion of adjustment of neurals and costals which forms the subject of Section 3.

## 1. OBSERVATIONS.

The first 243 specimens that could be observed carefully are included in Tables I to IV. These tables may, therefore, give a fair idea of the proportion of "abnormal" individuals in this species.

Table V includes a few specimens that were selected from a considerable number of others that have come under observation.

2. REVIEW OF OBSERVATIONS.<sup>2</sup>*Inframarginals.*

The *inguinals* are by far the most common scutes not typical of the shield of this species. No less than 21 per cent of the 243 specimens possessed one or both inguinals. In 33 individuals inguinals were present on both sides (v. Figs. D and E), but in 4 of these a distinct difference in size was noted between the scutes of the pair. Seventeen specimens had inguinals on only one side (Fig. F). *Axillaries* were wanting in only one terrapin, but were quite small in two others. In 5 specimens one axillary was wanting (Fig. L), and in 4 others one scute was at least twice as large as its mate of the opposite side (Fig. 4). Another individual (No. 114) had axillaries of normal appearance, but they were found to be loosely attached to the bone beneath, the skin connecting them with the adjacent scutes permitting a certain freedom of movement when the axillaries were pressed with the finger. It may be noted from the tables that a right inguinal occurred alone 12 times as opposed to 5 instances of the left alone, and the right was distinctly larger than the left 3 times as against a larger left once; also, while the right axillary occurred without the left 5 times, the left without the right was not noted. The right axillary was twice as large as the

<sup>2</sup>Numbers and percentages refer to Tables I to IV, unless otherwise stated.

left in 3 specimens, while the left was three times as large as the right in one terrapin. In Table V the left inguinal occurs twice without the right (Fig. F) and both are found in Nos. 254 (Fig. G) and 255. An additional scute in this "inframarginal series occurred in one or two specimens: in 254 and possibly in No. 233. (Figs. G and L.)

### *Interplastrals.*

The shield of *Malaclemmys* has normally no interplastral scute, and such a scute was found to be a very rare abnormality, as it occurred only in Nos. 75 and 181. In both cases it was small and

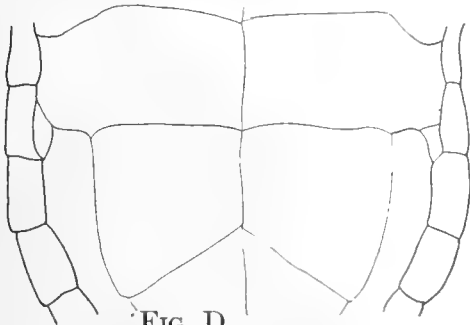


FIG. D.

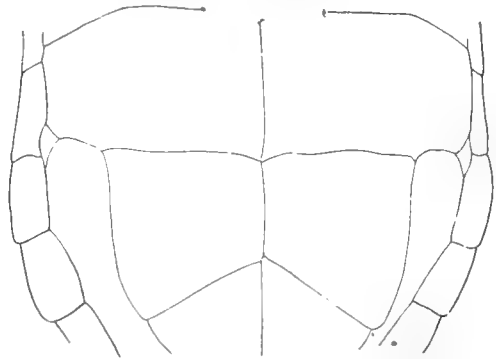


FIG. E.

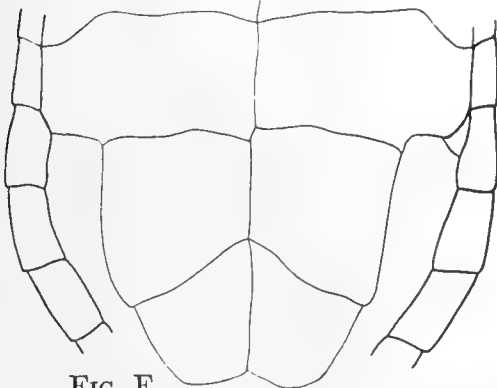


FIG. F.

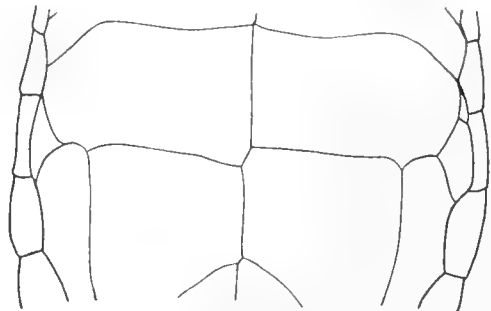


FIG. G.

FIG. D. Plastron of No. 149, showing unsymmetrical pair of inguinals.

FIG. E. Plastron of No. 161, showing paired inguinals.

FIG. F. Plastron of No. 253, showing left inguinal.

FIG. G. Plastron of No. 254, showing pair of large inguinals and a small scute anterior to left inguinal.

situated at the meeting-point of gulars and brachials (Fig. H, cf. Fig. B). In some species of turtles a large scute in this position is a normal feature of the shield (Fig. I). I do not know of any species in which characteristic interplastrals occur elsewhere. New-



mamm has observed interplastrals, as abnormalities, at other points, but always at the meeting-point of four plastral scutes.

### *Plastrals.*

The plastral scutes (cf. Fig. B) are remarkably constant; only two supernumerary scutes were noted. No. 132 showed an anomalous scute between the femoral and the anal of the right side (Fig. K), and in No. 233, two scutes occupied on the right side a space equivalent to that occupied by the femoral on the left (Fig. L). No. 88 presented an interesting abnormality discussed in a former paper ('05 a, pp. 14-18 and Fig. 6). In this specimen, the abdominal and femoral scutes were entirely separate to a certain point, but the last three rings of growth were perfectly continuous between the two scutes internally or mesially (Fig. M).

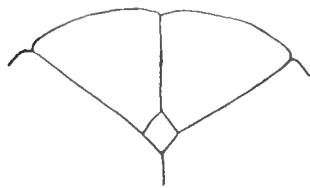


FIG. H.

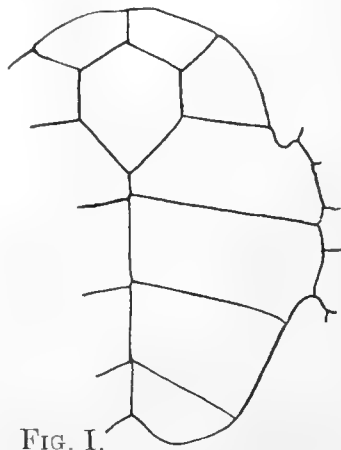


FIG. I.

FIG. H. Anterior portion of plastron of No. 181, showing small "inter-plastral."

FIG. I. Part of plastron of a species of *Chelodina*, showing large "inter-plastral."

In at least one species of turtle *Eretmochelys imbricata*, the scutes of the shield (Car. and Plas.) are imbricate and are said to be added to anteriorly as they wear away posteriorly. In the carapace of *Malaclemmys* the scutes are not imbricate, but they grow more in the anterior and lateral directions than in the posterior direction. In the plastron, however, there are often traces of overlapping posteriorly, especially in young shells. The posterior part of the rings of growth, usually very narrow, may be particularly so in such specimens, or even entirely wanting, the mesial segments of the

rings ending abruptly at the posterior margin which overlaps the next scute. The overlapping was particularly striking in No. 50.

No. 145 was characterized by marked asymmetry of abdominal and femoral scutes (Figs. 37, 38, Pl. IX).

### *Marginals.*

Except in two malformed shells, supernumerary marginals occur only in the regions anterior to the normal position of M 3, and posterior to that of M 10. This means that none occur except anterior or posterior to the region of the dorsal ribs. The exceptions are Nos. 145 and 248 (see Figs. 37 and 40, Pl. IX, and "Remarks" in tables). Anterior supernumerary scutes were found in Nos. 126 and 131 (Figs. 13 and 9). In each of these the first two marginals (R and L in No. 126, R in No. 131), taken together, are about equivalent to a normal first marginal. In No. 210 (Figs. 5 and 17) the first right marginal was partially divided.

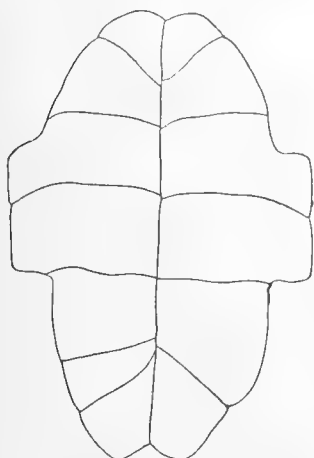


FIG. K.

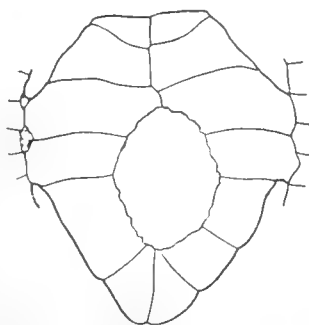


FIG. L.

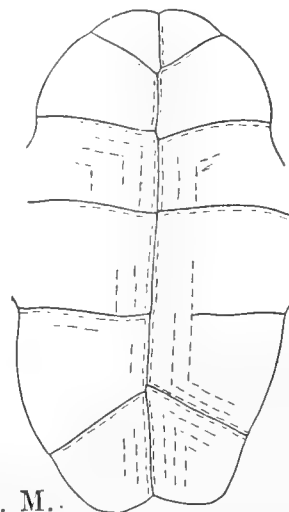


FIG. M.

FIG. K. Plastron of No. 132, showing anomalous scute on right side posteriorly. FIG. L. Plastron of No. 233, supernumerary scute on left side.

FIG. M. Plastron of No. 88, left femoral and abdominal continuous mesially.

Abnormalities are more common in the posterior region, additional marginals being observed on the left side in No. 235, on the right in Nos. 88 (Fig. 12), 129, 151 (Fig. 7), 183 (Fig. 33); on both sides in Nos. 35, 36, 37, 38, 108, 162, 178 (Fig. 30), 196 (Fig. 42), 198 (Fig. 45), 209 (Fig. 32), and 228 (Fig. 22); cf. also Table V, Nos. 250 (R), 252 (R), 255 (L) and 256 (L) and figures.

Sometimes the marginal series shows a reduced number of scutes. Thus Nos. 4 (Fig. 20) and 8 had only eleven pairs of marginal scutes, the eleventh marginals being very long. No. 245 had only eleven on the left side (Fig. 18) and the two posterior scutes of the left side, taken together, are equivalent in size to the last three on the right. Partial fusion of 11 and 12 (R) was noted in No. 47 and of 2 and 3 (R) in No. 176 (Fig. 34). In Nos. 153, 174 and 185, the twelfth marginals were reduced in size, and, in the latter two specimens, were asymmetrical (Figs. 26 and 29).

### *Nuchal.*

The most interesting abnormalities of scutes are those shown by the nuchal and the neurals. The most anterior of the median scutes of the carapace is always given the name *nuchal*, but is generally included with the other median scutes in the neural series. It resembles the other neurals in being median and usually unpaired, but differs from them in some evident respects. Its direction of predominant growth is the reverse of that of the other median scutes. It is absent in some existing species and in the fossil turtles of the genus *Pleurosternum* Owen, belonging to the sub-order *amphichelydia* Lydekker, a group which von Zittel regards as ancestral to the modern Pleurodires and Cryptodires ('02). I find it occurring in paired condition or marked with a median furrow much more frequently than any other commonly unpaired scute of the carapace. In this connection the frequent evagination of this scute in *Clemmys guttatus* is of interest.

The nuchal was represented by a pair of scutes in ten specimens: Nos. 44, 74, 78, 97, 124, 168, 196 (Figs 31), 200, 210 (Figs. 5 and 17), and 228 (cf. also Table V, Nos. 244, 245 and 249, and figures). A distinct median furrow, not distinguishable as a seam, occurred in nine individuals: Nos. 3, 73, 116, 125, 182, 185, 208, and 211; a faint median furrow was observed in No. 80.

### *Costals.*

It will be observed that abnormal scutes occur much more frequently in the posterior costal region than in the anterior.

Typically, each costal series consists of four scutes, which dovetail between the five neurals. The seam between C1 and C2 meets the fifth marginal near its anterior end, and the succeeding costal seams meet alternate marginals (seventh, ninth and eleventh). These typical relations of the costals to the shields of the other series, may aid in the diagnosis of supernumerary schutes (v. Fig. A).

In Fig. 9 the first large scute in the L. costal series shows essentially the relations of the normal first costal, so that the small scute anterior to it may fairly be considered the supernumerary element. Such anterior supernumerary costals may occur on both sides, approximately symmetrically (Nos. 244, 245, and 246, Pl. I, Fig. 2, and Pl. VII, Figs. 18 and 19); or on both sides but quite asymmetrically (No. 218, Pl. IX, Fig. 41), or on only one side (No. 9, right side, Pl. II, Fig. 3, and Nos. 4 and 131, left side, Pl. VII, Fig. 20, and Pl. V, Fig. 9). In each case referred to above, the extra scute was small in comparison with the other costals. In No. 218 supernumerary scutes were observed both anteriorly and posteriorly (Figs. 41 and 44).

Perhaps some of the cases to be discussed below belong in one of the above classes. In Nos. 210 (Figs. 5 and 17) and 151 (Figs. 7 and 15) the supernumerary scute in the costal series may be the large C1; but it would be difficult if not impossible to determine in these instances whether the first or second scute is to be regarded as supernumerary. We must always recognize the possibility that in some cases we are not presented with the four normal scutes as individuals plus an extra, "supernumerary," individual, but simply with a five-scute series instead of a typical four-scute series. Furthermore it may be quite immaterial whether an anomalous scute is termed a neural or a costal; the designation may be without real significance. Of course, however, if supernumerary scutes are attributed a morphological value, their proper classification is of the first importance (cf. below, p. 42).

The difficulty of identification of scutes is enhanced in the posterior region, where it is often difficult to decide whether a given anomalous scute is to be classed as a neural or a costal [cf. Nos. 210 (Figs. 5 and 17) and 252, Fig. 49]. It was found necessary to adopt certain rules of classification which may be in some measure

arbitrary. They are based on the fact that the seam between C4 and N5 typically meets M11 at a point near the middle or anterior part of its inner border (Fig. A, etc.).

1. (a) In a specimen with the usual number of twelve marginals, any scute in order with the costals which does not extend positively beyond M11 is considered a costal. This rule certainly becomes arbitrary when applied to small scutes which extend neither anteriorly nor posteriorly to M11, and which may, therefore, be as much within the neural region as within the costal region (Pl. VII, Fig. 21). It is convenient, however, to class such elements with the costals.

Compare:

much within the neural region as within the costal region (Pl. IV,

- No. 56, Pl. VII, Fig. 21, small scute, right side
- No. 133, Pl. V, Fig. 10, small scute, left side
- No. 167, Pl. VII, Fig. 16, large scute, left side\*
- No. 217, Pl. X, Fig. 46, large scute, right side†
- No. 218, Pl. X, Fig. 44, medium scutes, both sides
- No. 250, Pl. XI, Fig. 50, small and medium scutes, left side
- No. 251, Pl. VII, Fig. 14, medium scute, left side
- No. 255, Pl. IX, Fig. 39, small scute, right side
- No. 256, Pl. VII, Fig. 23, medium scute, right side
- No. 257, Pl. VIII, Fig. 27, LC4 represented by two scutes

(b) Sometimes extra marginals occur in association with extra costals; in such cases, the costal region may be supposed to extend to the twelfth marginal, which is the next to last, as is the eleventh in the typical series. Therefore, if there are thirteen marginals, a scute in order with the costals, which does not extend posteriorly to M12, is classed as a costal.

Compare:

- |                                   |                          |
|-----------------------------------|--------------------------|
| No. 151, Pl. { IV } Fig. { 7, }   | large scute, right side* |
| No. 196, Pl. { VII } Fig. { 15, } | medium scute, left side  |
| No. 198, Pl. IX, Fig. 42,         | small scute, each side   |
| No. 255, Pl. X, Fig. 45,          | small scute, left side   |
| No. 256, Pl. IX, Fig. 39,         | small scute, left side   |
| No. 256, Pl. VII, Fig. 23,        | small scute, left side   |

2. If, in a specimen with twelve marginals, a scute in order with the costals extends barely over on M12, but in its anterior

\*In the left costal series of this specimen, the supernumerary costal may, of course, be one of the more anterior scutes.

†No. 217 is incorrectly included in this list. It may properly pertain to the next list (b), but is a very doubtful case.

part is in contact with M10, it may fairly be regarded as a terminal costal that encroaches slightly on the neural region.

Compare:

No. 195, Pl. III, Fig. 8,	medium scute, left side
No. 199, Pl. X, Fig. 43,	medium scute, left side
No. 210, Pl. { III, } Fig. { 5, }	medium scute, left side*
and, possibly,	
No. 181, Pl. X, Fig. 47,	medium scute, left side
No. 149, Pl. IV, Fig. 11,	large scute, left side

3. A scute which overlaps the last marginal and is not in contact with any marginal anterior to the next most posterior (M11, in typical marginal series), is within the neural region and must be classed as a neural.

Compare:

No. 222, Pl. X, Fig. 48.
No. 252, Pl. XI, Fig. 49.
No. 256, Pl. VII, Fig. 23, small scute on right side.

It is doubtful whether the extra scutes on the left in Nos. 149 (Pl. IV, Fig. 11) and 181 (Pl. X, Fig. 47) should be included in this or the preceding class. The small scute on the right in No. 247 (Pl. VIII, Fig. 24) would seem to belong in the first class, but the shape of the areolæ of this and of N5 and its manner of growth as indicated by the rings suggest clearly that it is but the separated lateral end of N5.

It may be that this scheme of classification, as applied to such specimens as No. 198 (Pl. X, Fig. 45) and 256 (Pl. VII, Fig. 23) is quite arbitrary. This leads to the general question whether the reference of many of the abnormal scutes to normal series has any other merit than that of convenience of description. This question will be referred to in a later section, but it is well to make clear the difficulty of identification because, if abnormal scutes are to be regarded as of morphological value, rational and exact classification is essential.

The difficulty of diagnosing the costal series of cases like No. 151 (Figs. 7 and 15, right side), 167 (Pl. VII, Fig. 16, left side) and 210 (Figs. 5 and 17, both sides) has been referred to. In No. 210,

\*Cf. footnote (\*) preceding page.

for example, the seam between the second and third shields of the right costal series, meets M5, and this is the usual position of the seam between normal C1 and C2. Considering this seam as corresponding to the normal seam between C1 and C2, we note that the following costals (C3 to C5 in this shell) have exactly the relations to the marginals normal for costals 2, 3 and 4. We have only to assume that normal C1 is represented by two scutes in this carapace, or that one of the first two scutes is supernumerary; the right costal series is thus made perfectly intelligible by considering its relations to the marginal series, and disregarding its relations to the neural series. Turning to the left costal series, the four large scutes present a perfectly normal appearance, and have nearly normal relations to the lateral apices of the neural series; yet not one of the four has normal relations to the marginals, though the marginal series of each side is nearly normal. This series would be intelligible if we disregarded the marginal relations. Either series is interpretable, provided we do not apply the same criteria to both sides. It is clear that the exact identification of supernumerary scutes must often be impossible or quite arbitrary. The significance of such cases will become clearer after the following section.

#### *Neurals.*

Excluding the nuchal, for present purposes, the neural series consists typically of a row of five median scutes, which dovetail laterally with the costals. The first four usually bear marked prominences, which together form a low serrated keel. The fifth, sometimes, especially in younger specimens, shows a very low and inconspicuous continuation of the keel (cf. Pl. VI, Figs. 12 and 13). This keel is suggestive of the serrated dorsal keel of the tail of *Chelydra*, and Hay and Newmann regard the neural scutes as serially homologous with the scutes of the dorsal keel of the tail of *Chelydra*. In view of the accredited phylogenetic significance of the keel prominences they may aid in the interpretation of supernumerary scutes in the neural series. Such scutes are almost always placed not symmetrically, in longitudinal sequence, or alongside of each other, but asymmetrically and wedged in together. The photographs



are most instructive. In Fig. 8, Pl. III, there are at least seven elements in the neural series, of which the most posterior five fit in together as if crowded out of a condition of longitudinal sequence, each scute extending across the median line. It will be noted that while the rings of growth indicate that each scute has grown most in the antero-external direction (toward the antero-lateral costal), yet each scute shows comparatively broad rings toward its antero-mesial neighbor of the same series, although growth in this direction must cause further distortion of the keel by pushing the prominences further and further away from the median line. Nevertheless the prominences still make a continuous but crooked carapace keel. No. 151 (Fig. 15) presents a different condition. Numbering the scutes from N1 posteriorly, (cf. Pl. IV, Fig. 7, of the same carapace) N3 and N5 are almost parallel in position to N4, and the keel of N5 extends anteriorly half-way by the keel of N4, to which it is exactly parallel. Clearly, in this case the keel of the carapace branches and is double for a part of its course.

No. 150 (Pl. IV, Fig. 6) may offer a clear case of longitudinal sequence, though the supernumerary scute is largely on the left side. No. 139 had a very similar anomalous element, but entirely to the left of the median line and without the keel prominence.

Nos. 167 (Pl. VII, Fig. 16) and 251 (Pl. VII, Fig. 14) are comparable to the first illustration given above.

In the cases cited, which are representative of a number of others, the keel prominences throw no definite light on the question at hand. Is it to be assumed that such scutes are primarily in longitudinal sequence but are crowded out of position? Or that they are really paired scutes, asymmetrically placed? Or is their significance something still different? Newmann has decided in favor of the first explanation, but suggests that Gadow would probably consider such types as evidence of the original paired character of the neural row.

It is possible *hypothetically* to regard many of the asymmetrical neural anomalies as illustrating only a *secondary* asymmetry, a *modified* longitudinal sequence; but some cases can hardly be referred to such a condition, especially when a portion of the keel parallels another portion, as in No. 151 described above. Now, is

the keel necessarily a single structure which we must not expect to find divided? So far as I know, there is no phylogenetic evidence of the neural series of turtles ever having been paired, or, of a double dorsal keel in primitive turtles (cf. Newmann, '56, p. 92). If we must regard these abnormalities as atavisms, we can hardly conceive of the atavism taking so often the form of paired supernumerary neurals; we would be led to assume, with Newman, that we were presented with a modified longitudinal sequence. Now, disregarding atavism for the moment, we will consider the anomalies as we find them.

No case of unmistakably paired neurals has been observed in the terrapins of North Carolina or Maryland, if we exclude the nuchal, which is, however, usually included in the neural series; but there are interesting abnormalities that are significant in this connection. The nuchal, as has been seen, is sometimes paired, sometimes marked by a median furrow in the position of the seam, and the furrow may be so marked as to make it difficult to distinguish from a seam, or there may be a seam on the anterior half of the scute, continued posteriorly by a furrow (*Thalassochelys*, Pl. XIII, Fig. 89, and Pl. XIV, Fig. 94). Apparently the difference between the furrow and the seam is that the furrow divides incompletely, while the seam divides the scute completely as far as the seam extends. Now in four specimens of *Malaclemmys* (Nos. 73, 78, and 211; also No. 244 of Table V), some of the neurals were marked by a median furrow similar to the furrows observed on nuchals, and in each of these cases the nuchal was either marked with a furrow, or paired. Especially suggestive is No. 70 (Pl. I, Fig. 1) where N2 shows a short furrow on the anterior rings,<sup>3</sup> and N4 has all of its rings intersected anteriorly by a seam. Compare also Pl. I, Fig. 2, of a terrapin with nuchal paired and with furrows on N1 and N2.

In logical sequence with these specimens come some terrapins (*M. littoralis*) from Texas. In a small number of specimens placed by Mr. W. P. Hay in the United States National Museum, we observe all stages from very incomplete to complete division of the

<sup>3</sup>Owing to the manner of reflection of light the furrow on N2 is not apparent in the photograph, though very evident in the shell.

neural series (Figs. 53 and 54).<sup>4</sup> (See also W. P. Hay, '05,

<sup>4</sup>These specimens are of peculiar interest on account of the precise median longitudinal division of neural scutes (partial or complete)—a rare abnormality, though common, apparently, in the specimens from this particular region.

NINE SPECIMENS OF MALACLEMYS LITTORALIS HAY.

No.	Length of plastron in inches.	Median longitudinal division of scutes, as follows:
258	4 $\frac{5}{8}$	N2 with seam complete except in anterior portion of areola (oldest portion of scute), and in first (most posterior) part of anterior ring of growth. N3 similar to N2, except that seam, anteriorly, arises a little later.
259	4 $\frac{3}{4}$	Nuchal paired. N1 partially divided posteriorly. N2 completely divided. N3 partially divided anteriorly and posteriorly. <i>Fig. 53.</i>
260	6 $\frac{1}{2}$	N2 and N3 divided except for areolæ. <i>Fig. 54.</i>
261	7	N1 with furrow anteriorly.
262	7	N3 partially divided anteriorly and posteriorly.
263	7	Nuchal paired. N2 divided except areola. N3 partially divided anteriorly and posteriorly.
264	7 $\frac{1}{8}$	N2 partially divided anteriorly and posteriorly. N3 divided except areola.
265	7 $\frac{1}{4}$	N3 slightly divided anteriorly—that is, just beginning to show division.
266	..	Only third and fourth neurals present; N3 marked anteriorly by faint furrow.

It appears that only a few of these scutes (possibly only N2 of 259, Fig. 53) were split at birth; some others (as N2 and N3 of 258) were divided only in the posterior portion of the scute (of course, the areola of the older stage represents the entire scute of the newborn turtle); while in others the division, anteriorly and posteriorly, appeared at various subsequent stages. In

regarding the abundance of such forms.) A young specimen of *Chelydra* in the zoological museum of the Johns Hopkins University has its keel marked with a distinct sagittal furrow which extends from the anterior margin of N1 to the posterior margin of N5, and is continuous through all of the keel spines except that of N5; such a furrow is seen less distinctly in the shells of older turtles.

In the light of these median furrows and incomplete and complete seams, and of the parallel keel prominences of No. 151, there would seem no inherent improbability of duplication of the median keel or of neural scutes, nor any necessity for explaining away appearances of duplication. To jump to the other conclusion, that the asymmetrical neurals are primarily paired scutes which are crowded out of a symmetrical plan, would be equally unwarranted. "Crowding" may be, at best, but a descriptive term referring only to the appearance presented, rather than to any organic phenomenon.<sup>5</sup>

May it not be that we have to do here neither with scutes in sequence nor with paired scutes, but with a real asymmetry of scute plan? Symmetry is a normal feature of the carapace, but the cases in question are admissably abnormal, and, on the face of it, the scutes are asymmetrically disposed. The question is thus: Is the visible asymmetry secondary and due to the crowding out of line of elements that are primarily symmetrical, or is there in such cases a real primary, though abnormal, asymmetry, which is perhaps correlated with some other asymmetry? The almost invariable association of asymmetry in number or arrangement of the costal scutes is strongly suggestive of the latter conclusion. Granting some primary abnormality arising either as a germinal variation or in consequence of environmental conditions, it may be that the conditions of growth cause the development of the neural scutes neither in linear sequence nor in pairs, but in essentially such an asymmetrical plan as is presented by the cases in question.

these nine terrapin fifteen neural scutes, besides nuchals, show some degree of division. Hay states that the longitudinal division of scutes was "so common that it was really difficult to pick out a full-grown specimen which did not show it in some degree."

<sup>5</sup>Extra marginals may be small or large but do not seem to be "crowded out," and the same may be said of extra costals.

The asymmetrical neurals lead us to the subject of the inter-adjustment of neurals and costals, a subject of sufficient significance to justify its treatment in a separate section.

### 3. ADJUSTMENT OF NEURALS AND COSTALS.

In the typical carapace, neural scutes and costal scutes have an alternating relation. The neural series dovetails on each side into the costal series and a neural seam extends transversely from the apex of each costal scute. Extra scutes may occur in different parts of the costal series and often only on one side. In cases where the abnormality is such as seriously to disturb the symmetry of the costal series, the adjustment of the neurals to the costals might be accomplished in one of several ways.

1. The sequence of neurals might remain normal, presenting some such appearance as is represented in Fig. N, I.<sup>6</sup> Such an arrangement of neurals without regard to the costals of one side has not been observed.

2. Neural scutes might have normal relations to costals on each side (cf. Fig. N, II). There would have to be one supernumerary neural in correspondence with the supernumerary costal, and some of the median scutes would occupy oblique positions. In such a carapace, the antero-posterior extent of a neural scute measured from the plane of its most anterior point to that of its most posterior point would be unusually great. The antero-posterior extent of N3 in the figure is indicated by the line  $x y$ . Disregarding temporarily some rare and partial exceptions, this plan of adjustment is not observed.

Asymmetrical costals often occur in such form as to give opportunity for plans something like one of the above, and these plans seem to offer the simplest schemes of adjustment for neurals. Since with the partial exceptions that will be mentioned later, neither of those plans is observed, we may assume that these hypothetical schemes do not accord with the laws of growth.

3. Assuming that a better coördinated carapace results when,

<sup>6</sup>In Fig. N the costal series is traced from an actual specimen (No. 151) and the same series is used in all three plans.

(a) on each side there is practically the usual relation between costals and neurals, and (b) neural scutes have not an antero-posterior extent that is relatively unusually great, we may imagine such a plan of neurals as is represented in Fig. N, III. On each side neural and costal scutes alternate in position, and the two series dovetail together in usual fashion, while cross seams so divide the neural area that no individual scute has an excessive antero-posterior extent. This plan is not a hypothetical one, as were the others, but is an inference from the observations. Compare with it the plan of scutes illustrated by Nos. 151 (Figs. 7 and 15), and 195 (Fig. 8).

The first two types of adjustment assumed above (hypothetical) may thus be defined:

1. Neurals with *normal adjustment* to costals on one side, but not on the other. Individual scutes show this plan very rarely.

2. Neurals with normal adjustment to costals on both sides, but with the two sides of the neural series related in a way that is rarely observed.

The third type (observed) may be defined thus:

3. Neurals with normal adjustment to costals on each side; the two sides of the neural series unsymmetrical and so related to each other that the plan of each side is largely restricted to that side.

In this manner of growth, then, we have a neural plan (or *half-plan*) on one side, a different neural plan on the opposite side, and each plan is adapted to the costal plan of the same side (cf. the diagram, Fig. O, III a. The mesial region of the carapace is supposed to be covered by a strip of paper). The plan of each side does not usually terminate abruptly in the median line as represented in the diagram, III, b (cf. however, Newmann's Fig. 6),\* but the scutes necessary to the plan of one side extend a little over the median line (III, c), sometimes almost or quite across to the opposite costals (III, d). In consequence, we get, not a plan on one side independent of the plan on the other side, but *on each side a plan that is more or less modified by the over-extension of the scutes necessary to the plan of the opposite side.*

\*Fig. P, XII.

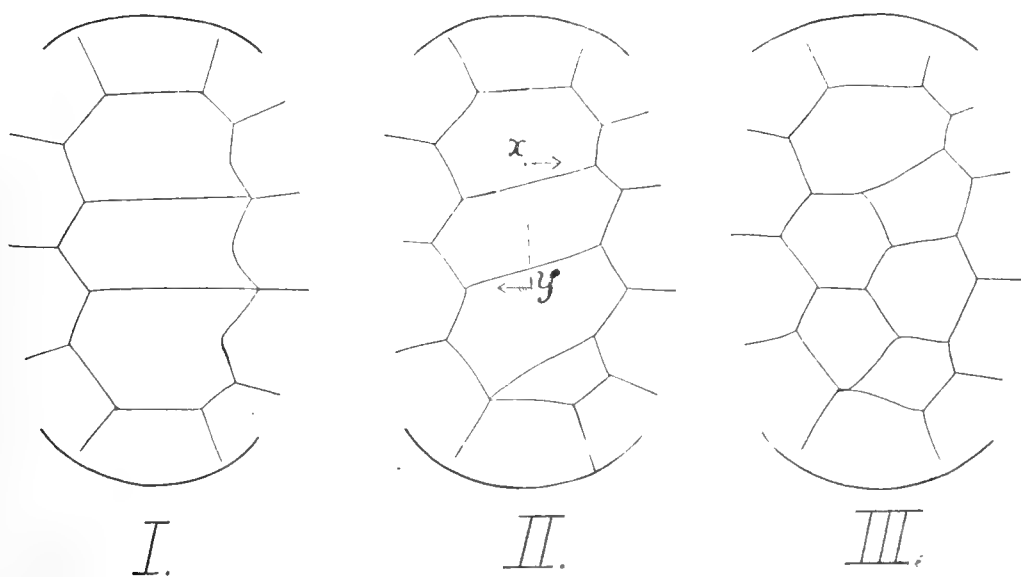


FIG. N. See text.

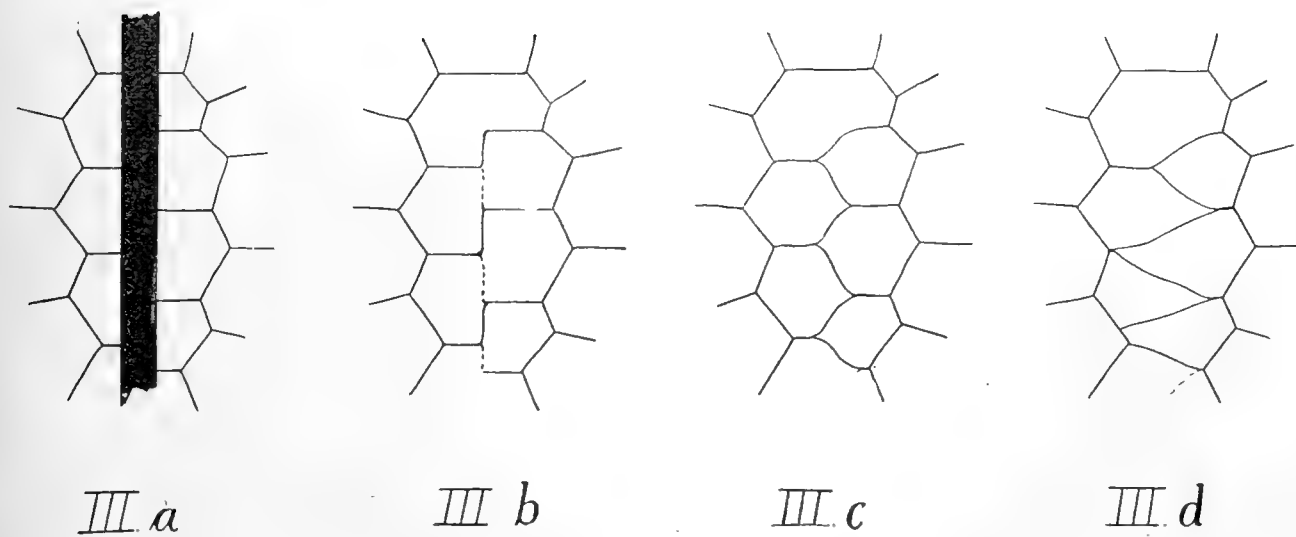


FIG. O. See text.



In the endeavor to make clear the inferred law of growth, I have, in a measure, proceeded in anticipation of the evidence from which the inference was made. The body of the evidence may now be best presented, not by detailed discussion, but by reference to text-figure P, in which are indicated the neurals and costals of a number of specimens. I have drawn freely on Newmann's figures, as his observations include more specimens bearing on this particular point than do my observations on *Malaclemmys*. My observations on another species need not be introduced at this point.

Each carapace figured may be compared, on the one hand, with the types Fig. N, I and II, and on the other hand, with that of Fig. N, III, or Fig. O, III c, and III, d.

It is readily observed that there is no correspondence between the *number* of extra costals and *number* of extra neurals, but that the *region of "abnormality"* in the neural series corresponds in antero-posterior extent with the region of *asymmetry* in the costal region. If asymmetry of costals is confined to a small region, the adjustment of neurals may require only one or two supernumerary elements (cf. Fig. P—V, VI, VII, etc.); but when asymmetry marks a larger part of the costal region the adjustment of the neural involves two, three, or more elements in excess of the normal number (cf. Fig. P—I, II, III, IV).

A few cases which may be classed as partial or complete exceptions require discussion. These are included in Fig. Q, to which refer the Roman numerals in the following paragraphs:

The carapace figured in VI illustrates in part the adjustment supposed. The adjustment would be complete only if a seam united the apices of RC4 and LC5. On any hypothesis this is a remarkably abnormal shield. (From Newmann's Fig. 9.)

In VII there are 5 costals on each side, but the number of neurals is normal. However, the *mesial* region of the two costal series are

<sup>7</sup>The nuchal and the marginals are omitted in most cases, since they have no bearing on the point in question, and would only complicate the figures. In the subscription full references are supplied, so that the original figures may be consulted if it is desired.

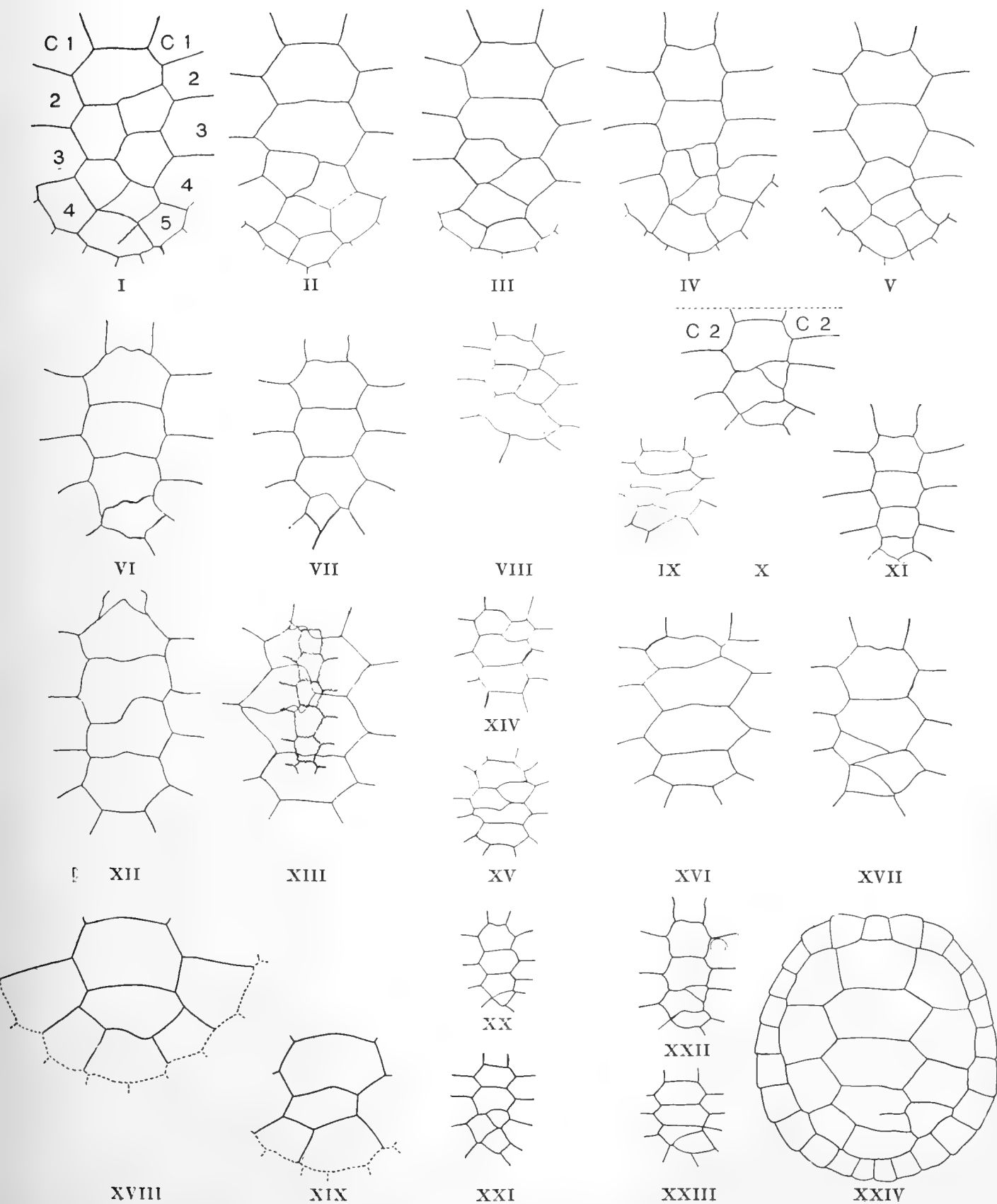


FIG. P. See text.

almost exactly symmetrical, and the second pair of costals are so small at their mesial ends as to be of little significance with reference to the neurals. It may be noted that the second neural is not longer antero-posteriorly than any other scute of the series. (From Newmann's Fig. 25.)

The four next following have an especial interest.

In I the adjustment appears in the posterior region, but more anteriorly are found two oblique neural elements. The most abnormal, N3, shows an *incomplete seam*, which, were it complete to the point *x*, would be a decided step toward perfecting the adjustment in the manner assumed. (Specimen No. 167, above.)

II presents a very complicated appearance, but the adjustment is more complete than at first appears. Thus, the two interrupted seams, *y* and *z*, are completed by furrows through the areolæ. The adjustment would conform perfectly to the usual plan, had these seams been complete, and had there been a seam in the position of the broken line *x*. The broken lines represent lines of depression of uncertain significance that radiate from the areolæ across the rings of growth (cf., the photograph of this carapace, Pl. II, Fig. 5). (Specimen No. 210, above.)

III represents a carapace that is very abnormal on any hypothesis. Even here, though, there is found an incomplete seam which makes a step in the direction of the assumed plan of adjustment. (From Newmann's Fig. 7).

In the carapace represented by IV, the "law of growth" in question would be fully expressed if the incomplete seam were complete to the point *x*. (From Newmann's Fig. 14.)

Recalling that the growth of the scutes is accomplished by the addition of peripheral rings, and observing that the incomplete seams are in each case in the peripheral or *newer* part of the scute, one may be justified in making the tentative inference that they indicate post-natal attempts to perfect a previously inadequate adjustment of neurals and costals. Certainly they *alter* the plan of adjustment, even if it be a coincidence that the alteration in these few cases is in the direction noted. This occurrence of apparently post-natal divisions in cases of imperfect correlation has been observed in

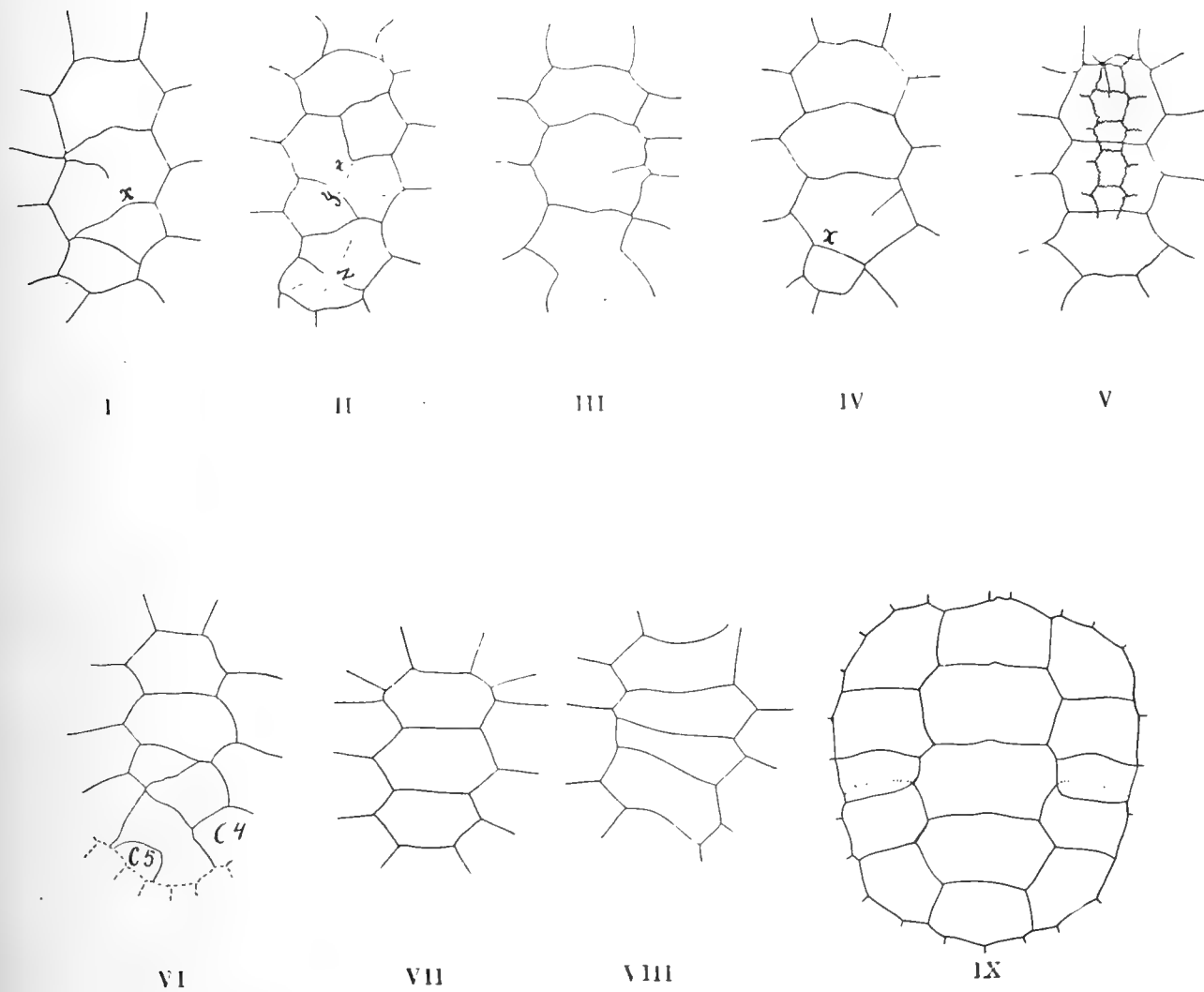


FIG. Q. See text.

the relations of marginal scutes and plates and will be seen again in embryos and young of *Thalassochelys*.

V violates the principle of adjustment in the anterior region. The second neural, exceptional in being in contact with three pairs of costals, is incompletely divided, but the significance of the incomplete seam is not evident. (Newmann's Fig. 4.)

VIII presents a very unexpected neural series; but this specimen was an embryo and we do not know how its co-ordination would have stood the test of life, nor whether post-natal seams would have appeared to alter the plan of adjustment. Several of the specimens mentioned above evidently would have shown less conformity if they had been observed in the embryo stage. (Newmann's Fig. 38.)

Another of Newmann's specimens is of particular interest (IX). The carapace is perfectly symmetrical, but there are five pairs of costals and no additional neurals. N3 is in contact with three pairs of costals (C2, C3 and C4). Here is, therefore, a case of mal-adjustment. However, Newmann found that the fourth costals were growing forward underneath the third, and he interprets this as a stage in the suppression of the third ("sixth," in his terminology). If this interpretation is correct, we have the mal-adjustment in process of correction, not, as in other cases, by the partial division of the neural, but by the *removal of the supernumerary costal*. I do not say, however, that the imperfection of adjustment is the cause of the squeezing off.

Hence the exceptions are chiefly partial exceptions, and, on the whole, even these specimens favor much more than they oppose the assumption that the proper *adjustment* of neurals and costals is of more vital importance than mere *number* of scutes. Of course, it is to be expected that turtles will show abnormalities in adjustment as well as in number of scutes; but observation of the relative frequency and degree of deviation from the usual numerical relations, on the one hand, and from the usual adjustment, on the other hand, may be a means of estimating the relative value of adjustment as compared with numerical relations. Supernumerary scutes occur comparatively frequently, while mal-adjustment is rare, and may it not be of some significance that in turtles with imperfect

adjustment, there is evidence of post-natal partial division of scutes by seams that *lessen* the abnormality in *adjustment* while they *increase* the abnormality in *number* of scutes?

There are two classes of costal abnormality that might seem to be exceptional and which, therefore, should be referred to here. (a) When small supernumerary elements occur at the extreme anterior or posterior ends of the costal series (Figs. 10, 21, 23, etc.), without interfering with the symmetrical plan of the normal scutes we would expect no change in the neural series—the adjustment of the series is already practically perfect. (b) Another illustration of asymmetry in *number only* is offered by the division of the 5th costal (Fig. 27). Two scutes may occupy just the position of this shield, without effect on the general plan of the series. On the other hand, of course, asymmetry of costals may occur without supernumerary costal scutes.

To sum up—

In the normal symmetrical carapace neurals and costals have an alternating relation, and the neurals dovetail between the costals. In the unsymmetrical carapace, this relation prevails on each side. It follows that one side of the neural series may be formed according to a different plan than that of the other side. The two opposing plans are independent of each other, but never entirely so. The scutes necessary to one plan extend more or less over into the other side, sometimes even to the opposite costal series, but always in reduced form. The two sides of the body show, at the same time, a degree of mutual independence, with a degree of mutual dependence.

In consequence of these conditions of correlation it is rare to find neural scutes of abnormally great antero-posterior extent, and there is noted a correspondence not between numbers of neurals and of costals, but between the respective regions of abnormality of the two series.

The value of these observations would be lessened if the suggestion of the general applicability of the principle inferred would conflict with previous observations, or with hypotheses which for other reasons we must accept. Previous observations on land and

marsh turtles have been used above); those on sea-turtles will be used in a later portion of this paper.

The only hypothesis which has a bearing is that which regards these abnormal scutes as atavisms. I believe that the general truth of these observations would conflict with that explanation, unless it could be supposed that the reversion was to a stage when neurals displayed the asymmetrical condition observed. In any event, I believe that the upholders of the hypothesis as applied to the scutes in question should account for the following facts:

1. That, generally speaking, more supernumerary neurals are observed in specimens with supernumerary costals on one side than in those with symmetrical supernumerary costals.

2. That, while supernumerary scutes may occur in any one series without additional scutes in any other series, thus indicating the partial independence of each series, yet, when a supernumerary costal occurs in such a position that the *symmetry* of the costal plans is seriously interfered with, the number of supernumerary neurals will depend on the extent of the region of asymmetry.

3. That we do not find asymmetry of costal plan without supernumerary neurals. (See qualification above, p. 41).

There is nothing in these observations to conflict with a supposition that the primary variation, the resulting adjustment of which we see, may be an atavism.

Referring again to the question of the identification of supernumerary scutes—as costals or neurals, etc.—if the adjustment of the whole carapace in accord with the laws of growth is the thing, it is of little real significance whether a given abnormal scute be termed “costal” or “neural,” however convenient such a classification may be for the practical purposes of description.

We have yet to consider the neural and costal scutes in relation to the bony plates beneath, but it is desirable to defer this until after the study of the scutes of *Thalassochelys*, which must conclude the present paper.

## 4. AGE, SEX, SYMMETRY.

*Age.*

It was shown in a previous paper ('05 a) that my observations do not indicate any significant difference in the proportions of abnormality at different ages. The cases of incomplete division and incomplete fusion noted all seem to tend toward increasing the abnormality in *number* of scutes. Newmann ('06), after examination of nearly 500 specimens of *Graptemys*, including a number of embryos, finds that "abnormalities are no more common in one size than in another." He notes, however, some cases interpreted as stages in the squeezing off of scutes (cf., my specimens No. 50, p. 22, 23).

*Sex.*

The proportion of abnormality in the females of tables I to IV is noticeably greater than that in the males. Considering all the abnormalities of carapace and plastron, as defined on p. 12, above, 24 out of 71 males are abnormal, or 34 per cent, and 71 of 135 females, or 52.6 per cent. Of 37 the sex is not known. Of the entire 243, 109, or 45 per cent are abnormal. If, however, we consider only the possession of more or less than the typical number of scutes in the carapace, the proportion of abnormality is about 20 per cent.

*Symmetry.*

51 specimens show only symmetrical abnormalities.

44 specimens show only non-symmetrical abnormalities.

14 specimens show both kinds of abnormalities.

Thus, of the 109 abnormal turtles of tables I to IV, 51 are symmetrical, 58 unsymmetrical, in the respect that we have taken into consideration.

Before taking up the embryos and young of *Thalassochelys* it will be well to give a summary of the observations on *Malaclemmys*.

## SUMMARY.

1. Observation of diamond-back terrapin in nature and in confinement reveals a marked degree of diversity in habit, disposi-



tion, and structure. Terrapins display much individuality in these respects.

2. The diversity in number and arrangement of the scutes accords with the general diversity. Of the first 243 specimens carefully observed, 20 per cent had either more or less than the typical number of scutes in the *carapace*. In all, 45 per cent showed such differences from the typical number, arrangement, and character of the scutes of carapace, plastron and inframarginal series, as to be classed for present purposes as "abnormal."

3. The "abnormalities" consist in: the possession of a greater or less number of scutes than 38 in the carapace, 2 in the inframarginal series, and 12 in the plastron; of instances of incomplete fusion or incomplete division of normal or abnormal scutes; of asymmetry in size and plan of scutes; of imbricateness of scutes; of the loose attachment of scutes to the bone beneath (one case).

4. *Asymmetry* manifests itself in various ways, and is at least as common in "abnormal" terrapins as symmetry.

5. *Females* show a much greater degree of diversity than *males*. This is true as regards both percentages of abnormal individuals and degree of abnormality in the average individual.

6. No evidence was noted of difference in the proportions of abnormality in *young* and *old* terrapins, but the data are inadequate for definite conclusion.

7. The most variable scute is the *inguinal*. Typically absent, it was present, on one or both sides, in 21 per cent of the 243 specimens. Its approximately symmetrical occurrence was noted in 13 per cent of the 243. Rarely, one or both *axillaries* were wanting. Sometimes they were reduced in size or asymmetrical.

8. The most variable scute of the carapace is the *nuchal*. Always present, it was sometimes paired, sometimes marked by a median groove.

9. Omitting the inframarginal series, the *plastron* is far less variable than the carapace. Diversity manifested itself by the presence of extra scutes in the plastral series and of inter-plastrals at the meeting-point of gulars and brachials, by partial fusion of scutes, and by asymmetry. Each abnormality is of rare occurrence.

10. Except in misshapen specimens, supernumerary *marginals* seem to occur only anterior or posterior to the region of the dorsal ribs. They are more common in the posterior region. A reduced number of marginals results from the lack of one or a pair of the posterior scutes. The alternating relation of scutes and plates is well preserved in several specimens.

11. Supernumerary *costals* may occur at the extreme anterior or posterior ends of the neural series, without disturbing the general plan of the series, or they may occur in such a way that the plan of the series is altered. The two series of costals may be asymmetrical even when the number of costal scutes is normal.

12. *Neural* scutes may be partially or completely divided by a median seam. This abnormality, though rare in the terrapins of North Carolina and Maryland, is very noticeable in those of Texas (Hay).

13. Asymmetrical scutes in the neural series are of not uncommon occurrence. There is observed an adaptation between asymmetrical neurals and asymmetrical costals that strongly suggests that the explanation of these scutes is to be sought in the *adjustment* of scutes consequent on some more primary asymmetry. I do not regard them as belonging in linear sequence and crowded out of position.

14. I believe that the asymmetrical neural scutes cannot be regarded, individually, as *atavisms*. Their adaptation to an *unsymmetrical* carapace seems too clear to permit of explaining them by reversion to any earlier symmetrical plan of scutes.

15. In the asymmetrical scutes we may find an illustration of the fact that we may best interpret a single variation not by regarding it as an isolated unit, but by viewing it in its relations to the associated structures with which it helps to form a more or less well co-ordinated whole.

## PART II. THE SCUTES OF THALASSOCHELYS CARETTA (L.).

### 1. INTRODUCTION.

#### *Material.*

Only embryos and young were observed and these were obtained from eggs laid on the ocean beach near Beaufort. In studying the abnormalities it is important to know the conditions under which the embryos developed. The laying ground is inconveniently distant from the laboratory and the nests could be visited only by a sail and a walk on the beach that consumed the greater part of a day. The main object at the time was the collection of embryological material, and, as it was important to have the eggs conveniently accessible, it was necessary to remove them to artificial nests on the island on which the laboratory is situated. Another condition making it advisable to transplant the eggs was the difficulty of protecting the natural nests from depredation. Turtle eggs have a local value as food and are eagerly sought by fishermen. It was observed too that hogs root up the nests and destroy the eggs.

One nest was left undisturbed. A wire screen, placed over it and well under the sand, served to prevent the escape of the young turtles and to protect the nest from hogs. Traces of the nest were obscured as far as possible to prevent molestation by fishermen. The eggs hatched successfully (see Table VIII).

Most of the eggs were removed from the nests within two days after they were laid, usually on the following morning, and transferred in a bucket or box, in which they were covered with moist sand and seaweed. Usually care was taken to keep the eggs right side up. The eggs were then replanted either in artificial nests on the ground or in a sand-box or "incubator." The artificial nests in the ground were not successful. The soil, though chiefly sand, was of a different composition, and a higher temperature obtained than at the same depth on the beach. Other environmental conditions seemed unfavorable, and, in consequence, but a small proportion developed to a late stage. Unfortunately, the first observations indicated that nests in the ground would be more satisfactory than nests in an incubator,

so that most of the eggs were placed in the ground. Later it was found that a proper incubator would yield better results than the soil of the island.

The incubator used was very simple. It consisted of a box with four shelves, each holding 72 eggs. The shelves were large enough for each egg to be entirely surrounded by a small amount of sand. The light cushions of sand around and above the eggs served to maintain a comparatively uniform condition of humidity, while at the same time permitting the eggs to expand in size without crowding. This growth of the eggs, by the filling out and distension of the shell, commonly occurs to a greater or less extent during the development of the embryo. The sand was sprinkled with water as often as necessary. It was not necessary to apply artificial heat since the chief problem was to keep the temperature as low as it would ordinarily be at the depth of the natural nests. To obtain fairly uniform conditions of humidity and temperature, this box was placed within another box so much larger than the first that there was the space of 6 inches between the side walls (except on one side), the bottoms and tops, respectively, of the two boxes. The space between the two boxes was packed with moist sand. On the fourth side the inner box opened by a thick door containing a six-inch thickness of sand. Outside of this was the door to the outer box. Thus the inner chamber was well protected from such rapid changes of temperature or moisture as might take place outside. At the same time it could readily be opened at any time and one or more eggs removed without disturbance of the others. If the temperature seemed rising too high, moistening the outside of the box and the top layer of sand would, through evaporation, lower the temperature within a day; or, if necessary, the doors could be left open, when the evaporation would lower the temperature very quickly. In this way it was not difficult to keep a tolerably uniform temperature of 26° to 28° C.

The observations to be given below will at least suggest that the eggs of the loggerhead sea-turtle would lend themselves well to experimental study, by varying the conditions of incubation. During the past summer (1905), however, the experiments were made subservient to the obtaining of embryological material and it can-

not be said that this paper includes experimental observations of more than suggestive value. The observations given below were made on such of the embryos (and newborn) as were old enough for the scutes to be clearly distinguishable.

Before proceeding to the tables a word should be said as to the normal conditions of development.

*Observations on Conditions of Development.*

The loggerhead sea-turtle makes its nest in the region of Beaufort, at or near the base of the sand-dunes that line the beach at a short but varying distance from the water. The nest is subspherical, somewhat flattened on top, and packed with eggs usually to the number of 120-150. The top eggs are 12 to 15 inches below the surface, and, as the nest is 10 inches or more in diameter, the bottom eggs are 22-24 inches below the surface. At this depth, the bottom eggs may be below the level of the high tides, or as much as 4 feet above it, according to the elevation of the ground at the foot of the dunes.<sup>8</sup>

As the shell of the egg is soft and, in its new-laid condition, not completely filled, it displays a characteristic movable dent, like a rubber ball incompletely filled with air. In the course of development the contents increase in bulk and the shell becomes filled out and spherical: it may even be tightly distended. Agassiz says, "The older the egg the more distended does the shell appear." I have not found that the distension occurs invariably or to a uniform degree. The degree of distension varies with external conditions. Distension generally takes place, and it may occur to an extreme degree. With a large number of eggs massed together deep under the sand, the swelling of the eggs must cause great crowding and considerable interpressure. In one nest, for example, the eggs were so distended that upon a single puncture of a shell with a needle, a fine stream of fluid would squirt out a distance of several feet and the shell burst widely in the hand. Even though many of the eggs that failed to develop shrunk in size so as to be almost flattened

<sup>8</sup>See also my paper, '06, pp. 61 to 65.

against the sand, yet the pressure among those that developed was such that they lost their spherical shape and were somewhat flattened on the sides where they pressed against other eggs. Some of this flattening was lost before the photograph was taken ('06, Pl. XX., Fig. B). The possible influence of such a factor as this inter-pressure of eggs in the production of abnormalities of various kinds is not to be ignored. The indirect results of localized pressure on the developing embryo of other animals is well known, especially through such work as that of Spemann in the production of double-headed embryos and "cyclopean defects." It may not be a coincidence that two "cyclopean" embryos developed in the nest just referred to and that almost all of the turtles were abnormal in scutes, and some in still other respects.

#### *Explanation of Tables.*

There are four main tables (VI to IX). Table VI includes embryos from a single nest obtained in 1903. No further observations were made until 1905. Table VII includes the embryos and newborn from various artificial nests in the ground, and this table has several subdivisions (A-E) in order to keep separate the turtles from different original nests. In this way, the degree of diversity in turtles of the same brood may be noted. Table VIII is based on new-born turtles from a natural nest that was not disturbed. Finally, in Table IX are turtles which developed in the incubator, where crowding was provided against.

The tables have essentially the same form as those in the preceding part of the paper. The number of scutes in a series is indicated only where abnormal, or, if normal in number but abnormal in plan, the normal number is written in italics. The number of marginals, however, is always indicated since it cannot be said that either 12 or 13 is abnormal. Thirteen on each side, 12 on each side, or 12 on one side, 13 on the other—each of these plans is common. For brevity they are referred to as the 13-13 plan, 13-12 plan, 12-13 plan, or 12-12 plan, the number given first being in each case that of the left side. These plans may be seen, respectively, in Figs. 83, 58, 72, and 52.

The length is the full length of the carapace measured over the dorsal curvature and expressed in millimeters. The day of the embryo is indicated, when the embryo was taken from the egg alive, but the stage of development is perhaps best inferred from the carapace length, since the length of the incubation periods varies within wide limits (73, or less, to 90 days, or more). It was longer for eggs in the incubator than for those in the ground. "B" in the "day" column, indicates that the specimen was new-born.

## 2. OBSERVATIONS ON DIVERSITY.

The carapace differs from that of *Malaclemmys* in *shape*—it has a more aquatic form. It is broad in the anterior region, where the long stout swimming flippers are, and tapers to a comparatively narrow but rounded posterior end. The margin is indented over the anterior flippers, and sometimes slightly so over the posterior. As if in adaptation to the shape, with broad anterior end, the nuchal is very wide and the costal series terminates anteriorly in a small scute not represented in *Malaclemmys*, and others. There is often one more marginal in this region than is found in land and marsh turtles.

In Table VI are presented the observations on 34 embryos from the nest transplanted at the laboratory in 1903. Besides the fact of removal in the first instance, the conditions of development were otherwise abnormal. Only a small proportion developed successfully and most were removed from the nest during the period of incubation. Only one (No. 32) actually hatched.

Twelve of the 34 are abnormal in number or arrangement of scutes or in showing partial division of a scute. The abnormality frequently manifests itself in an asymmetrical plan of neural and costal scutes (Nos. 1, 23, 24, 26, 30, 31). In other cases the costal series of the two sides are unsymmetrical in number, without effect on the symmetry of the general plan of series (Nos. 6, 10, 21, 25). The proportions of these embryos with the several marginal plans referred to on p. 49, above, are interestingly uniform. Of the 29 specimens, in which the marginals could be counted with certainty, ten have the 13-13 plan, ten the 12-12 plan, and nine either 13-12 or

12-13. The most abnormal carapace is that of No. 26 (Fig. 63), with the large scute between neural and costal series, the small area in the mesial posterior region without a horny covering, and the minute scute that appears as if it were cut out of the left twelfth marginal. These three abnormalities are each unique among my observations.

The only striking abnormality noted, apart from scutes, was in No. 22, the fore feet of which were somewhat rudimentary.

Three small embryos of this nest are not included in the tables. One of these seems to have five neurals, a second six, and the third seven. The other scutes can not be distinguished with certainty.

Table VII, A, includes 21 embryos from two artificial nests, the eggs of which came originally from a single natural nest. In the table the first ten numbers are from one artificial nest. At the time of removal it was noted that the eggs were much distended, and, from the consequent crowding, more or less misshapen. One-half of the eggs started development, but not more than one-fourth were living on the 44th day. Two of the embryos were peculiarly abnormal. No. 36 shows an approach to the "cyclopean defect" (cf. 91, 92), but its scutes are normal. No. 35 (Figs. 66 and 67) besides having a harelip, was characterized by a remarkable foreshortening of the body; both heart and lungs are outside of the umbilical opening; the carapace, which measures 10 mm. in length and 17 in width, is almost regular and symmetrical, though its marked deficiency in length is accompanied by a corresponding reduction in the number of scutes in longitudinal series. As a transverse series of scutes may be said to consist of one neural, a pair of costals and two pairs of marginals, the shield is deficient by two complete transverse series, less two marginals on the right side. The inframarginal series are somewhat reduced, but the plastron has the full number of scutes, including an *intergular*, often wanting, and even one supernumerary scute posteriorly.

Three other specimens are abnormal in respect of scutes of the carapace, and one of these (No. 43) possessed minute "supra-marginals."

The last 11 numbers in the table are from a different artificial nest. Most of these eggs developed to some extent, but only half



(11 out of 24) were living on the 49th day. The striking feature of this small group of embryos is that more than half displayed small scutes just above the marginals and always at the meeting point of three scutes (Figs. 73, 74). For convenience these are termed in the tables "supramarginals," though it is not intended thereby to imply any homology between these anomalous scutes and the typical supramarginals of *Macroclommys*. The prevailing plan of marginals is 13-13.

Table VII, B, is based on 14 embryos from a nest transplanted at the laboratory. Less than one-third of these eggs developed. Four of the 14 are abnormal, in having supernumerary marginals (three specimens), a symmetrical supernumerary neural (No. 60), or two scutes in the place of normal RC5 (No. 66). The marginal plan, 13-13, prevails. The large number of marginals (15-14) in No. 60 is noteworthy.

Table VII, C, includes 2 embryos and 7 new-born turtles from a nest transplanted at the laboratory. Three are abnormal, two of these having the costal series more or less asymmetrical and, in correlation with the costals, asymmetrical neural scutes. In one specimen (78) two neural scutes, in another (73) three are not completely separated from one another.

Table VII, D. This is a rather remarkable collection of embryos. Of the 22 specimens 18 are "abnormal," and these include several that are abnormal in scutes to a high degree.

Four are markedly deformed. No. 93 (Fig. 89) is asymmetrical and somewhat misshapen, and the number of marginals on the right side is unusually small. Two of these (Nos. 91 and 92) have an interesting deformity of the head. We are not concerned in this place with the details of anatomy, but the external characters of these specimens may be sketched briefly. The anterior mesial region of the face is reduced. The eyes are thus brought close together, or fused, and are enclosed by single upper and lower eyelids. The single nostril opens at the end of a short snout above the upper eyelid. In one specimen the snout points anteriorly. In the other it is, as it were, rolled back on the top of the head, the nostril appearing on the dorsal side of the posterior end of the flattened

backward pointing snout. In accord with the reduction of the parts just above it, the upper jaw is much shortened and flattened from the front so that it extends across from side to side in a more direct way than usual. The lower jaw, however, has about its usual form; so that, instead of being, as usual, enclosed within the upper jaw, it protrudes well beyond the upper. A somewhat similar embryo has already been alluded to (No. 36), Table VII, A). All three are further characterized by the very slight development of pigment.

The most remarkable deformity noted is that of No. 100 (Figs. 93 and 95). The lower jaw forms a pointed horizontal projection from the ventral part of the head, and on the dorsal aspect of this projection is a longitudinal slit representing the mouth. About halfway up the anterior aspect of the head is another pointed projection (snout?). There is no external evidence of eyes. The body is characterized by a reduction of its dorsal part. The carapace consists of two scutes with a smaller ovoid scute anterior to these (Fig. 95). On each side a bridge of one scute connects the carapace with the plastron, which possesses the full number of scutes. Posteriorly the plastron (Fig. 93) bends sharply dorsally in consequence of the reduction of the dorsal region. The dotted line in the figure indicates the region of the angle. Between the upper posterior end of the plastron and the posterior end of the carapace is the tail bearing the anus on its dorsal aspect. The heart, lungs, stomach and intestine lie external to the umbilical opening. The limbs are situated dorsally.

Among the smaller embryos with scutes undeveloped and, therefore, not included in the table was a fifth deformed embryo from the same nest. It needs only an allusion here. The body is reduced, and rounded, and the limbs appear rudimentary.

In view of the excessive proportion and degree of abnormality observed in this lot of embryos, the conditions of development should be described. The original lot of eggs was brought to the laboratory by a fisherman. According to his statement they were a mixed lot from two nests taken the preceding day. Some of them had dried somewhat and the lot was regarded as unpromising. However, they were placed in a single nest on the island made as usual in imita-

tion of a natural nest. The spot chosen proved to be rather more moist than usual. During development the eggs swelled to an unusual degree. As a result they were very much crowded and were misshapen (cf. remarks above, p. 48-49).

Many of these abnormalities are such as we would naturally attribute to the abnormal conditions of development, and the question naturally suggests itself: can the conditions of development be responsible for the remarkably large proportion of abnormalities of scutes in this nest?

The abnormalities take these forms chiefly:

1. Costals symmetrical in number, asymmetrical in plan, neurals asymmetrical (88, 97), or symmetrical (92).

2. Costals asymmetrical in number and plan, neurals asymmetrical (82, 90, 94).

3. Costals asymmetrical in number (through absence of normal small first costal), symmetrical in plan neurals symmetrical (80, 81, 87, 98, 99). Also, LC5 represented by two scutes (91).

No. 82 is further abnormal in that the first costal forms part of the margin (Fig. 84).

The nuchal is frequently paired or furrowed. The marginals are usually 12-12.

One specimen possesses supramarginals (No. 88, Fig. 85).

Table VII, E. The eggs were brought to me by a fisherman, who stated that they were a mixed lot from two nests taken two days before. They were replanted in the ground. The fourteen embryos and new-born make a comparatively normal lot. Though more than half (8) are abnormal, the variations are comparatively slight, consisting chiefly of paired nuchals, supernumerary costal posteriorly, supernumerary neural posteriorly, or incomplete division of N4 or 5, and slight asymmetry in the anterior region (104, 107). The marginal plan 12-12 is most frequent.

In Table VIII are presented the observations on 76 turtles from an undisturbed natural nest (see p. 46, above). The young turtles were found under the wire some days after hatching (88th day from the beginning of incubation). Only one unhatched egg remained in the nest.

As there were very few abnormal turtles, and since all are of approximately the same age, the table may conveniently be abbreviated. First the abnormal turtles are listed, and then the normal turtles in several classes according to the plan of the marginals.

The abnormalities are: C5 represented by 2 scutes (4 specimens), nuchal paired (119), N5 incompletely divided transversely (120), and, supernumerary marginal (121).

All of the common marginal plans occur, but 13-13 largely predominates (40 specimens).

As the nest was never disturbed during the period of incubation (except at the start) it cannot be stated whether or not there was much swelling of eggs with consequent pressure between them. This undisturbed natural nest yielded a far greater proportion of normal turtles than any nest transplanted into the ground at the laboratory.

Table IX. On account of the possibility of the pressure in natural and artificial nests having some effect on the method of growth of the scutes it was desired to remove some eggs from such conditions. Therefore, a number of eggs from two or three nests were placed on the shelves of the incubating box described above. The eggs were surrounded and covered by a very small amount of moist sand, but not sufficient to offer any considerable resistance to the expansion of the egg in any direction. On account of the poor success of the eggs transplanted into the ground, the incubator was frequently drawn on for embryological material, especially to fill the gaps in the earlier stages. Table IX, therefore, includes only 18 embryos—all from a single original nest. The number is entirely inadequate for conclusion, but it was interesting to find that only a single specimen was abnormal (by the possession of a 6th neural—cf., Fig. 79). The marginals display exceptional uniformity. In the three youngest specimens they were not distinct, in one the plan is 13-12, but in *all* the others the plan is 12-12. It is possible that the eggs from this original nest would have developed with as much uniformity under other conditions.

The table can conveniently be abbreviated.

TABLE VI.

THIRTY-THREE EMBRYOS AND ONE NEWBORN.

Eggs from one original nest transferred to one artificial nest in the ground.

Serial Number.	Length of Carapace in Millimeters.	SCUTES.								Figures.
		Nuchal.	Neurals.	Costals.		Marginals.		REMARKS.		
				Left.	Right.	Left.	Right.			
1	23	1	5	5	5	?	?	(Normal).	57	
2	24					?	?	(Normal).		
3	25		x			13	13	N5 partially divided transversely.		
4	26					?	?	(Normal).		
5	26					?	?	(Normal).		
6	26	x		4		12	13	Marginal series encroaching on nuchal.		
7	27					?	?	(Normal).		
8	27					12	12	(Normal).		
9	27					13	13	Normal.		
10	28			6	4	12	12			52
11	29					12	12	Normal.		
12	29					12	13	Normal.		
13	30					13	12	Normal.		56
14	30			6		13	13			
15	30			x		13	12	Normal except that LC1 is rather large at expense of LC2. LM2 very small.		
16	31					12	12	Normal.		
17	31		x			12	12	N5 divided obliquely; seam distinct on R side, faint on L posteriorly.	55	
18	31					13	13	Normal.		
19	31					12	13	Normal except R marginals. Cf. figure.	59	
20	31					13	12	Normal.		
21	32			4		13	12	Supn'y marg. on left side posteriorly.		
22	32					12	12	Normal. (Fore-feet reduced).	61	
23	33		7	6		13	13			
24	34		8			13	12			62
25	35			x		13	13	L costal series normal except for minute scute at posterior end.	60	
26	36	pr	6	x	x	12	12	Difficult to diagnose; cf. figure..		
27	39	f	x			13	13	Faint furrow on N1 and N2, extending into spine; abrupt back side of spines furrowed.	63	
28	39					12	12	Normal.		
29	40					12	12	Normal.	64	
30	44	pr	7	4	6	13	13	Faint furrow on N1 and N2 and, more distinctly, on N3, Supn'y marginal posteriorly on left side.		
31	45		6	6		13	13			65
32	46					13	13	Normal. Nuchal and N1 and N2 show very faint traces of furrow. (Hatched.)		
33	48					13	12	Normal.		
34	52					13	13	Normal.		

TABLE VII.

EMBRYOS AND NEWBORN—FROM ARTIFICIAL NESTS IN THE GROUND.

## A. 21 Embryos—Originally from One Natural Nest.

Eggs transferred to two artificial nests in the ground. The first 10 embryos below (Nos. 35-44) came from one of these nests, the remainder from the other.

SCUTES.										
Serial Number.	Length of Carapace.	Day.	Nuchal.	Neurals.	Cos- tals.		Marginals.		REMARKS.	Figures.
					Left.	Right.	Left.	Right.		
35	10	44th		3	3	3	8	10	Remarkable foreshortening of body; compare figures. Width 17 mm. (Head with hare lip defect.) (Plastron with one supn'y scute.)	66 67
36	19	"					12	12	Scutes <i>normal</i> . ("Cyclopean defect" of head.)	
37	19	"		7		5	12	12	RC3 much reduced.	71
38	23	"					13	13	<i>Normal</i> .	
39	24	"					13	13	<i>Normal</i> .	69 72
40	24	"					13	13	<i>Normal</i> .	
41	25	"					13	12	<i>Normal</i> .	69 72
42	26	"				5	13	13	RC5 incompletely divided.	
43	26	"		7		5	12	13	RC4 somewhat reduced, so that RC5 occupies a position more anterior than normally—cf. relation to marginals. Minute supramarginals observable with lens.	70
44	28	"					13	13	<i>Normal</i> .	
45	36	49th		6		7	13	13	2 scutes occupying place of RC5; also 1 supn'y scute posteriorly.	73 74
46	37	"					13	13	<i>Normal</i> .	
47	40	"					13	13	1 supramarginal, left side, between C2 and M8.	73 74
48	41	"					13	12	3 supramarginals on each side; one is minute.	
49	41	"					13	13	<i>Normal</i> .	75
50	41	"					14	13	Supramarginal between C2, C3, and M8, right side.	
51	41	"					13	13	Minute supramarginal between C3, C4, and M8, left side.	75
52	42	"					13	13	<i>Normal</i> .	
53	43	"					13	13	Small supramarginal, between C3, C4, and M8, left side.	75
54	48	"		5			13	13	N5 is completely divided transversely.	
55	50	"					13	13	2 supramarginals on R side, 3 on L. Fig. would apply to R side, with smallest scute omitted; applies to L side.	

## B. 14 Embryos.

Originally from one natural nest.

56	35	?					13	13	Normal	80
57	38	45th					12	13	Normal.	
58	41	"					13	13	Normal.	79
59	41	"					12	13	Supn'y marginal posteriorly, right side. Malformation of posterior margin right side.	
60	41	"		6			15	14	Regular scute following N4. Marginals with 14-13 plan anteriorly but with supn'y scutes posteriorly, each side.	68
61	42	"					13	12	Normal.	
62	43	"					12	13	Normal.	76
63	43	"					13	13	Normal.	
64	44	"					13	13	Normal.	68
65	44	"					13	13	Normal.	
66	45	"				6	13	13	RC5 represented by 2 scutes.	68
67	46	48th	x				13	13	Normal, except that nuchal is notched and shows slight median furrow.	
68	46	"					13	13	Normal.	76
69	48	"		6	6		14	14	LM2 and LM4 very much reduced.	

TABLE VII.—(Continued.)

C. 2 *Embryos* and 7 *Newborn*.

Originally from one natural nest.

Serial Number.	Length of Carapace.	Day.	SCUTES.						REMARKS.	Figures.
			Nuchal.	Neurals.	Cos- tals.	Marginals.				
						Left.	Right.			
70	53	63d	x			13	12	Normal. Trace of median furrow on nuchal.		
71	53	"			6	13	13	RC5 represented by 2 scutes. RM2 much reduced.	83	
72	49	B				12	12	Normal.		
73	49	"		x		12	12	Neural series of 7 elements, but 3 of these (N4, 5 and 6) are incompletely separated from one another.	81	
74	51	"				12	12	Normal.		
75	52	"				13	12	Normal.		
76	53	"				13	12	Normal.		
77	53	"				12	12	Normal.		
78	55	"	x	x	x	13	13	Neural series of 8 elements, but N3 and N4 are not completely separated mesially. Nuchal with distinct furrow to left of median line, anteriorly. LC1 wanting; LC5 represented by 2 scutes.	82	

D. 22 *Embryos and Newborn.*

Eggs originally from 2 natural nests—but transplanted into one artificial nest.

79	20	?				12 ? 12	(Normal.) L marginals not thoroughly clear anteriorly.	
80	29	54th	pr		5 6	12 12	Supn'y costal on each side, but LC1 wanting. No supn'y neural, but N4 with beginning (?) division on each side so as to give usual adjustment.	78
81	32	"		5	6	13 13	Symmetry of LC1 and 2 with RC2 and 3 suggests that there is a supn'y costal on each side while LC1 is wanting.	77
82	34	"	x	6		12 13	Very abnormal: see text, p. 54.	84
83	35	"	x	6	5 4	12 12	Normal. Slight median furrow on nuchal posteriorly.	
84	35	"	pr			12 12		
85	38	?	pr			12 12		
86	40	54th				12 12	Normal.	
87	41	"				12 12	RC1 merged in 2.	
88	41	?	x		4	12 13	Nuchal divided anteriorly. N6 showing trace of seam, distinct on left posteriorly. 5 "supramarginals", 4 of which are very minute.	85
89	44	54th	f	6		12 12		
90	51	"			6	12 12	Much of the distortion observable in the figure is due to contraction in the preservative.	86
91	47	73d	pr	7		12 12	LC5 represented by 2 scutes. "Cyclopean".	88
92	48	"	x		6 6	12 12	Scutes abnormal anteriorly. "Cyclopean".	87
93	52	"	x	5		12 10	Somewhat asymmetrical and misshapen. Nuchal with median seam anteriorly, continued posteriorly by a furrow.	89

TABLE VII.—(Continued.)

Serial Number.	Length of Carapace.	Day.	SCUTES.						REMARKS.	Figures.
			Nuchal.	Neurals.	Cos- tals.		Marginals.			
					Left.	Right.	Left.	Right.		
94	53	"		8	6	12	13	N2, 3 and 4 incompletely separated mesially.	90	
95	54	"	pr			12	12			
96	52	B				12	12			
97	53	B		8	6	6	13	13	<i>Normal.</i> Supn'y scute in each costal series. Probably LC6 is supn'y, since the costals anterior to it have the usual relations to marginals. On the right side the scutes posterior to RC3 have the usual relations to marginals of C2—5; hence RC2, or 3 is probably supn'y (contrast with Nos. 80 or 92).	91
98	53	B				4	12	13	RC1 wanting, LC1 reduced. Supn'y marginal posteriorly, right side.	92
99	54	B				4	12	12	Like the above, except for the supn'y M.	
100	6	54th							Remarkable carapace of 3 scutes. v. text, p. 53. Plastron with normal number of scutes.	93 95

## E. 14 Embryos and Newborn.

From one artificial nest. Eggs from two natural nests mixed.

101	31	38th						12	12	<i>Normal.</i>	
102	33	"	x					12	12	Nuchal divided symmetrically by a seam anteriorly, continued as a furrow posteriorly.	94
103	35	"		5				13	13	N4 partly divided transversely.	96
104	39	"	x	x	x	x		13	13	Asymmetry of nuchal, first neural, first costal and first marginals.	98
105	40	47th						12	12	<i>Normal.</i>	
106	41	"	pr					12	12		
107	42	"	pr					13	13	Nuchal seam oblique.	97
108	46	B	x					12	12	<i>Normal.</i> Trace of furrow on nuchal.	
109	47	B		6				12	12		99
110	48	B						13	12	<i>Normal.</i>	
111	49	B						12	12	<i>Normal.</i>	
112	49	B	pr					14	13		103
113	50	B						12	13	<i>Normal.</i>	
114	51	B		5	6	6		12	12	N5 partly divided. LC5 represented by 2 scutes. Supn'y costal on R side.	101



TABLE VIII.

76 NEWBORN TURTLES FROM A NATURAL NEST.

Serial Number.	Day.	SCUTES.						Figures.	
		Nuchal.	Neurals.	Costals.		Marginals.			REMARKS.
				Left.	Right.	Left.	Right.		
115	B	pr	5	6		13	13.	LC5 represented by 2 scutes.	102
116	"			6		13	13	LC5 represented by 2 scutes.	
117	"			6		12	12	LC5 represented by 2 scutes.	
118	"				6	13	13	RC5 represented by 2 scutes.	
119	"					12	12		
120	"					13	13	N5 divided on R side	
121	"					13	14		
122 }	"					13	13	36 <i>normal turtles.</i>	
157 }	"					13	12	8 <i>normal turtles.</i>	
158 }	"					12	13	13 <i>normal turtles.</i>	
165 }	"					12	12	12 <i>normal turtles.</i>	
166 }	"								
178 }	"								
179 }	"								
190 }	"								

TABLE IX.

EIGHTEEN EMBRYOS AND NEWBORN.

Transplanted from a single natural nest to an incubator.

Serial Number.	Length of Carapace,	Day.	SCUTES.						REMARKS.
			Nuchal.	Neurals.	Cos-tals.		Marginals.		
					Left.	Right.	Left.	Right.	
191 } 193 }	12	32d	?				?	?	3 embryos <i>normal</i> as far as observable.
194	12	34th					12	12	<i>Normal.</i>
195	13	35th					12	12	<i>Normal.</i>
196	17	34th		6			12	12	Supn'y neural posterior.
197 } 200 }	x	34th						12	<i>Normal</i> embs. Car. of 196 and 197: 19 mm.
									Car. of 198 and 199: 20 mm.
201	47	60th					12	12	<i>Normal.</i>
202 } 207 }		B					12	12	6 <i>Normal</i> turtles.
208		B					13	12	<i>Normal.</i>

## 3. REVIEW OF OBSERVATIONS.

*Marginals.*

In strong contradistinction to most turtles there is no definite number of marginals that can be considered normal. In the 12-plan (see above, p. 49), the first costal is in contact with only two marginals (M1 and M2), the second with four (M's 2, 3, 4 and 5)—Fig. 74. In the 13-plan, the first costal is in contact with three marginals, the second with M's 3, 4, 5 and 6. The differences between the two is therefore, the presence in the one case, the absence in the others, of a scute between M1 and the marginal opposite the seam posterior to C1 (marked *Mx* in Figs. 76 and 79). This scute varies in size from a very minute scute entirely surrounded by the scutes preceding and following it (Fig. 76) to a size as large as the others, Fig. 79 right. Compare Fig. 79 left, Fig. 76 right, and Fig. 83, left and right. Sometimes, with the 12-plan anteriorly, there occurs a posterior scute, making the number really 13. This is spoken of as the 12-plan with a supernumerary scute posteriorly.

Sometimes an extra marginal appears in the region of C2. This scute, *My*, Fig. 76, leads to a 14-plan if *Mx* is also present. Again, a scute, *Mz*, may intervene between the most posterior marginal and the marginal opposite the seam posterior to normal C5. In this event the series contains 13, 14, or 15 scutes, according as the 12-plan, 13-plan, or 14-plan prevails anteriorly. Compare Fig. 92 right—13 scutes (*Mz*), Fig. 79, right—14 scutes (*Mx*, *Mz*), Fig. 79, left—15 scutes (*Mx*, *My*, *Mz*).

As to the relative frequency of the common marginal plans, 12-12 and 13-13 occur approximately in equal proportions; 12-13 and 13-12 occur each about one-third as often as either of the symmetrical plans. The scutes *My* and *Mz* are of rare occurrence. Supernumerary scutes at other places were not observed. The stippled area of M10, Fig. 84, represents an infolded area. The posterior limb is folded over the carapace in this region, and the slight malformity of the margin noted in this region in this and other specimens (Figs. 80 and 76) undoubtedly results from undue pressure of the limb against the margin, and this suggests the same explanation for the malformed margins of *Malaclemmys* (Figs. 32, 37, 39, 40).

Such crude direct effects of pressure are not to be confused with the possible indirect adaptive results suggested above (p. 49).

Less than 12 marginals were observed only in malformed specimens. In No. 35 (Fig. 66), with marginals 8-10, the right marginal series shows less reduction than any other series of the carapace. In No. 93 (Fig. 89) the marginals are 12-10.

#### *Supramarginals.*

Small scutes, just above the margin and at the meeting-point of three scutes, are noted only in No. 88 of Table VII D (Fig. 85), and in several embryos of Table VII, A (Nos. 43, 47, 48, 50, 51, 53 and 55, Figs. 73 and 74). The costals do not seem quite to meet the marginals in early stages, and if the presence of scutes between costals and marginals be attributed hypothetically to arrest of development, these observations may lend some support to Newmann's hypothesis as to the number of primary series of scutes in the carapace. But would not such an assumption as to the significance of supramarginals place them in a distinct class from other supernumerary scutes for which arrest of development is not to be hypothesized?

#### *Nuchal.*

As was the case in *Malaclemmys*, so here the nuchal is frequently found in paired condition (eleven instances, Fig. 103, etc.), or marked by a median furrow, which may be quite distinct (three instances) or very faint (four instances). In one case an anterior seam was continued posteriorly by a furrow (No. 93, Fig. 89).<sup>\*</sup> The furrow is incomplete in No. 78, Fig. 82, and the seam incomplete in 88, Fig. 85. The division is usually symmetrical but not always so.

#### *Costals.*

Each costal series consists of five scutes, the most anterior being very small, and having no analogous element in *Malaclemmys*. It may be noted that, when there are twelve marginals, the first two costals are in contact with the first five marginals. In *Malaclemmys*, which has, typically, twelve marginals, the first costal is in contact

<sup>\*</sup>Also No. 102, Fig. 94.

with the second to fifth marginals, the narrowness of the nuchal bringing the first marginal into a position anterior to the first neural. Where an anterior supernumerary costal occurs in *Malaclemmys*, the relation of the first two costals to the marginal corresponds to that in *Thalassochelys*, Fig. 9, left. Sometimes the small first costal of *Thalassochelys* is wanting (seven instances) and then the condition approximates that of *Malaclemmys* (cf. Figs. 57 and 92). Symmetry in this abnormality was never noted; in fact, in two of the seven specimens a supernumerary scute was present on the opposite side, Figs. 52 and 64. This suggests that the absence of this scute is attributable to some primary asymmetry rather than to reversion.

Another not infrequent abnormality of costals consists in the presence of two scutes in the place of C5. The two scutes, together, have practically the usual relations of C5 to marginals on the one side and to neurals on the other. Cf. Figs. 70, 83, right, and 56, 82, 88, left. Nos. 10, 66, 115, 116, 117, 118, 114 left (Figs. 52, 101) have the same abnormality. Contrast with these the posterior supernumerary scute of No. 69, Fig. 76, and 114, right, Fig. 101, also the incomplete division of LC5 in No. 42 (Fig. 69).

The abnormalities of costals so far mentioned, while manifesting asymmetry so far as the numbers of scutes on the two sides goes, leave the two series symmetrical in general plan. But the plan is disarranged in such specimens as Nos. 94 (Fig. 90) and 97 (Fig. 91) and, as we shall see below, the number of extra neurals occurring in association with such abnormalities of costals depends on the extent of the asymmetry.

Asymmetry may occur without supernumerary costals, Figs. 71, 72, etc.). On the other hand, supernumerary costals may occur symmetrically; Fig. 78 illustrates this, if it be assumed, as seems apparent, that LC1 is wanting while the next to last costal of each side is supernumerary.

#### *Neurals.*

Paired neurals or neurals incompletely divided in the median line were not found, but a slight median furrow was sometimes observed.

A very symmetrical supernumerary neural sometimes appears in sea-turtles just posterior to N4. This scute is almost perfectly

rectangular (Fig. 79). Such a shield was found in two of four shells of *Colpochelys kempi*, and in three of thirty-one specimens of *Chelone mydas*. In several cases there is a horizontal seam confined to one side, which, if complete, would isolate such a scute. (Nos. 3, 54, 103, 114, 120, Figs. 75, 96, 101 and 102.) Hence the seam which cuts off this scute from N5 occurs more frequently on one side than on both. The partial independence in variation shown by the two sides is nicely illustrated in this case, as in many others.

A seam may start from the usual point of origin of the seam bounding the rectangular scute posteriorly, but bend posteriorly to meet the last costal distally or the marginals (Nos. 37, 73, 109, 196). Cf. Figs. 71, 99, and 81. A short seam completed by a furrow is shown in Figs. 55 and 85.

In Fig. 96 N4 is partly divided by a seam on the right side which is completed on the left by a furrow.

#### *Adjustment of Neurals and Costals.*

No. 81 (Fig. 77) illustrates well this adjustment: neurals and costals in alternating relation. In this specimen I infer that LC1 is wanting and that a pair of supernumerary scutes is present; but, disregarding this inference, there are, on each side, five costals in contact with neurals posterior to N1, while in the normal carapace there are only four. As the costals are nearly symmetrical from this point posteriorly, a single nearly symmetrical extra neural serves to maintain the usual alternating relation. In 31 (Fig. 65) the supernumerary costal is on the left side alone, and here again we find on each side the usual alternating relation of neurals and costals. Counting down the left side there are six neurals, counting down the right side there are five. The supernumerary neural is not absolutely restricted to the left side nor unmodified on that side, but tapers to a point. It seems that in *Thalassochelys*, the asymmetrical scutes are not in general as much restricted to one side as in *Malaclemmys* and *Graptemys*. Shells, such as No. 94 (Fig. 90) and 97 (Fig. 91) are very suggestive of the types noted in the first part of this paper (p. 34, above), but more commonly the asymmetrical elements are of the type of those of 43 (Fig. 72) and 90 (Fig. 86),—

comparatively broad on both sides but widest on the side where they are in proper alternation with the costals. The subject has been so fully discussed in a previous section that I need only refer to the figures: Figs. 61, 62, 64, 72, 70, 84, 85, 90, 91, 100, and a few others that need special discussion.

In No. 80 (Fig. 78) there is the normal number of neurals with, evidently, a pair of supernumerary costals; N4 is a large scute where we would expect two scutes to complete the alternation; but we find on each side the beginning of division opposite the apices of costals.

In No. 210 (Fig. 104) the incomplete seam on the left side is to be noted.

In 73 (Fig. 81) the correlation is complete except in so far as two of the seams are incomplete mesially.

No. 78 (Fig. 82) presents an unexpected scute in the oblique fourth neural. The incompleteness, mesially, of its anterior seam suggests that this scute was still more abnormal at an earlier stage. But if this carapace is to be in harmony with the others, we would expect the development of a seam from the point *x* extending toward the left end of the third neural or toward the second costal.\*

No. 94 (Fig. 90) has already been referred to; but it is to be noted that two of the seams are completed in the mesial region only by furrows.

No. 92 presents a clear case of "mal-adjustment" (Fig. 87). N2 is an exceptionally large scute and there are not even the beginnings of seams from the apices of the third costals. It is not surprising, however, that this turtle should fail to manifest the usual laws of development in scutes or in any other organs. Its "cyclopean defect" alone gives it a hopeless deformity.

#### *"Incomplete Division."*

It may be said that it is "begging the question" to assume that the incomplete seams represent *division* rather than *fusion*. Against the latter explanation it may be said that such seams are always in the peripheral or growing region of the scutes, and that, where

\*The letter is omitted in the plate. The point *x* is on the fourth neural seam near the apex of the third right costal.

they shade off into furrows, the furrow is mesial, and the seam most distinct at the periphery. Further, in *Malaclemmys*, as has already been shown ('05a, p. 14 ff.), the successive concentric rings of growth give a sure means of determining that the incomplete seams noted in that species are of subsequent development. This seems sufficient to make "partial division" acceptable. It may be added that the incomplete seams would be quite inexplicable on the supposition of fusion. Gadow's hypothesis may presuppose fusion of scutes, but these cases could not fall in line with his hypothesis.<sup>9</sup> We would have scutes fusing that were not supposed by the hypothesis to fuse (cf. Figs. 81, 82, 91). On the other hand, as expressions of laws of growth that are not complied with until these seams appear, the late appearance of the seams is quite explicable. It is evident that, instead of suggesting arrest of development as the explanation of supernumerary scutes, they would point in a contrary direction. If they have a significance as to ontogenetic development they point to perfection of adjustment as opposed to perfection of number of elements. See also Fig. 104 (specimen 210, not listed).

Finally, whether the blind seams represent division in process, as suggested, or merely broken seams in stable condition, their positions and their peculiar association with other abnormalities are too suggestive to go unremarked.

We speak of supernumerary *scutes*, although it may be more a matter of *seams* than of scutes. We have to do merely with the subdivision by seams of a given horny area. What determines the plan of the seams, to what extent it is dependent on vasculature or on the relations to the bones and other organs beneath, we do not know, but it is quite conceivable that the laws of growth and of adaptation to diverse environmental conditions are adequate to account for the diverse plans of scutes. The evidence points very directly to the conclusion that the development of asymmetrical seams in the neural region takes place in accord with laws of growth as distinguished from laws of heredity, and this may well be the case with other abnormalities.

<sup>9</sup>Gadow's figures are not reviewed here, since, though showing a high degree of multiplication of scutes in the several series, the supernumerary scutes are hardly comparable to those dealt with in this section; supernumerary scutes

Salient features of the observations on *Thalassochelys*:

(1) The very high proportion of abnormality in these turtles, which developed chiefly under abnormal conditions.

(2) The general unsymmetrical aspect of the abnormalities, the marginal, Mx, being an exception, since it occurs on both sides more frequently than on one side alone.

(3) The occurrence of small "supramarginals"—not previously recorded as an abnormality; these are always at the meeting point of three other scutes.

(4) The development of an embryo with only 3 scutes in each dorsal series (Fig. 66), and another *with 3 scutes* forming the entire carapace.

(5) The appearance of a number of "monstrosities"—"cyclopean" embryos, etc.; and the association of a high proportion of abnormality of scutes with the occurrence of these monstrosities.

Supernumerary scutes occur in the neural and costal series with much irregularity and without symmetry. The only symmetrical recurring scute in either series is the rectangular scute posterior to N4. At least two abnormalities in the costal series show a regularity in their recurrence: these are—the absence of the small C1, and the presence of two scutes in the place of C5; but neither of these abnormalities appeared on both sides and symmetrically.

### PART III. SIGNIFICANCE OF THE ABNORMALITIES.

#### *Introduction.*

The specimens of *Thalassochelys* observed display an even greater degree of diversity in number and arrangement of scutes than did those of *Malaclemmys*. While Newmann found that one-tenth (48) of 476 specimens of *Graptemys* had supernumerary scutes in the carapace, and only about one-twentieth of 188 specimens of *Chrysemys*, about one-fifth of the first 243 specimens of *Malaclemmys* observed had more or less than the typical number of scutes. But

are intercalated, generally in association with intercalated costals, sometimes independently, but not with marked asymmetry. In fact, the only abnormalities in my specimens that resemble those of his are the presence of 2 scutes in place of C5, and the interposition of a small neural posterior to N5.



in 208 specimens of *Thalassochelys*, almost one-third were abnormal. The diversity in *Thalassochelys* is even greater than this proportion indicates, for, in estimating that proportion of abnormality, both twelve and thirteen marginals were considered as normal. The variable M2 is absent almost exactly as often as it is present and in one-fourth of the total number of specimens it is absent on one side while present on the other. It seems remarkable that there should be such diversity in forms that have been so little modified through several geological ages. *Thalassochelys* is at least as old as Eocene times, and turtles of the Upper Jura have essentially the same number and arrangement of scutes as have modern turtles. From one point of view the arrangement of scutes is exceptionally plastic or variable, while from another, the paleontological point of view, it is exceptionally persistent and fixed.

It would be interesting to know the explanation of the "abnormalities," such a large proportion of which show asymmetry, but the data that we have in hand are, comparatively, very scant. It is advisable, however, to consider the explanation advanced hitherto (atavism) and other possible explanations in the light of the data in hand.

#### *External Conditions.*

We have not sufficient evidence as to the influence of the environmental conditions of the eggs during development in the production of abnormalities of scutes. A large proportion of abnormalities was found in each of the nests transplanted into the ground at the laboratory, all of which were under more or less unfavorable conditions. The nest which showed by far the largest proportion of abnormal specimens was one in which very great pressure from the distension of the eggs was noted. The smallest per cent of abnormality yielded by any nest in the ground was found in the turtles from eggs left undisturbed to hatch under natural conditions; but the most normal lot of turtles of all was that obtained from eggs which developed in the "incubator," with the element of pressure eliminated. The series is inadequate for positive conclusions, however, as the number of embryos is too small, but it suggests the importance of further experiments.

*Inheritance from Immediate Ancestors.*

The diversity in some of the lots of turtles, each of which groups came from one original nest (Tables VI and VII, A, B, and C) certainly does not suggest that the abnormalities of the parents had a great influence on the abnormalities of the young, but on this point, again, the data are inadequate.

*Atavism.*

It has been seen that the prevailing interpretation of the abnormal scutes is that they are atavisms; "reminiscences of earlier, phylogenetic conditions" (Gadow, '05, p. 638); "examples of systematic atavism in the sense of de Vries" (Newmann, '06, p. 69). In studying this diversity of scutes, then, one is confronted at the outset by these questions: are all of the abnormalities to be regarded as atavisms? and, if not all, which anomalies are due to reversion? Comprehensive use of the conception of "systematic atavism," as applied to these variations, has been made for morphological purposes, and as the observations given in this paper might be taken some to confirm, some to modify, others to negative such morphological views, I consider it necessary to inquire with some fulness regarding the basis of the atavistic interpretation of supernumerary scutes.

It has already been made clear that asymmetrical neurals are not to be regarded as instances of reversion. Further, from a glance at such figures as Figs. 63, 66, and 95, we infer that some others of the abnormalities are surely not referable directly to atavism. But, are most of the supernumerary scutes to be explained by reversion? and, *by what criterion shall it be decided if a given scute is atavistic or coenogenetic in character?*

By *atavism* we understand ordinarily the reappearance of a character, not manifest in the immediate ancestors, but typical of some remote ancestral form. It is assumed that this character, though disappearing from view, has never been actually lost, but, having assumed a latent condition, has been transmitted from generation to generation until finally, on the proper occasion, it reasserts itself. Atavism is, therefore, much more than a descriptive term referring

to the resemblance of a new to an old character; it is a positive assumption of the unbroken continuity of the old quality in latent condition through succeeding generations and its final reappearance as a variation.

The need for this theory of reversion has been felt as an explanation of the observed fact that the offspring often manifests some character not possessed by its immediate parents, but peculiar to its grandparents or to some more remote ancestors. Especially in the latter case do we, in Darwin's words, "feel a just degree of astonishment," and, whether the resemblance is to a near or to a more remote ancestor, does there seem a need for the theory of latent characters and reversion. It would seem, however, that the theory was one to be invoked only in cases where there is strong reason to believe that the resemblance is not superficial or accidental, but so vital and unmistakable as to be explicable only as a direct inheritance. Yet the theory of reversion has been overworked to the point of being applied to cases of the most superficial resemblance, even to variations that bear resemblance only to a purely constructive ancestor, the hypothetical existence of which is based largely on the arbitrary assumption that the anomaly in question is an atavism.

The subject of polydactylism in higher vertebrates not only offers an excellent illustration of the wholesale use of the theory to explain all kinds of anomaly, but it is one which has been so much longer and more thoroughly studied in its various aspects (anatomy, heredity, etc.) than has the subject of supernumerary scutes, that we may be allowed to draw some lessons of caution from its history, if we do not seem to imply that the same principles must apply to scutes as to digits.

Darwin himself, "with much hesitation," attributed polydactylism in man to reversion, but soon retracted ('76, p. 459), with the explanation that he had been misled by the statements of observers. The bifid rays of Selachians, and "constructive" 7-toed ancestral mammals, have been called up to account for modern polydactylism (Albrecht, Bardeleben, etc.). Other writers, as Gegenbaur, and, especially, Bateson and Prentiss have put the matter in a better light. Certainly the following simple principles, self-suggesting, would seem demonstrated in regard to polydactylism.

1. External resemblance is not ground for the assumption of reversion (since the presence of two digits in the horse is sometimes due perhaps to the development of the vestigial second digit, or, in other cases, clearly to the duplication of digit three. (Prentiss, '03, p. 299).

2. Recurrence of a similar abnormality in numerous individuals of the same or different species is not ground for the assumption of reversion. (Cf. duplication of hallux or pollex in man, cat, dog and fowl. Bateson, Prentiss, etc.)

3. Where extra elements occur which are, presumably, of vestigial origin and possibly attributable to reversion, the atavism may be by no means true to the ancestral condition reverted to; for Prentiss ('03, p. 291) finds that, in a large number of cases, the supernumerary *vestigial* digit appears in a condition of partial or complete duplication—that is to say, in a condition directly misleading if used for inference as to ancestral form.

4. Supernumerary elements have a negligible value as basis for phylogenetic hypothesis.

Without regard to polydactyly, the above considerations suggest themselves *a priore* against the attribution of definite morphological significance to supernumerary scutes. I refer to polydactyly because these *a priore* objections seem to gain weight from their demonstrability in another field of variation.

In regard to scutes the following points may be noted:

1. We have no evidence as to the value of external resemblances—as to whether the same appearance might not be due in one individual to one cause, in another to a different cause.

2. The assumption that anomalies of scutes are atavisms rests on the recurrence of certain more or less characteristic scutes in different individuals of the same species, and in different species, and the correspondence of such scutes in a few instances to normal scutes of still other species. But recurrence of certain very definite anomalies in the same or in different species is often noted in cases where atavism would not be suggested. Thus the cyclopean defect occurs in man, and in an essentially similar form in amphibia (Spemann, '04). I have had apparently the same sort of defect to

appear in three embryos of the loggerhead turtle, taken from artificial nests, and the resemblance to the same abnormality in man and in Triton is striking. Here is the same abnormality occurring in different and widely removed species, and with remarkable definitions of form.

The matter of recurrence need present little difficulty. If the organization of one turtle is much the same as that of another of the present time or of a considerable period of past time (and observations so indicate), I do not know why turtles may not be subject to similar anomalies; nor why there may not occur now the same variations that once, in connection with others, characterized the ancestors of a diverging species; nor why, if any of the variations now occurring should characterize a future subspecies or species, they might not continue to occur in other turtles without acquiring a new significance.

3. The use of the supposed atavisms as morphological data implies not only the transmission of the primitive characters in latent condition from generation to generation since remote geological ages, but also that they are now seen practically in pure form and unmixed with any appreciable number of new inheritable variations, occurring first during these ages since the primitive characters became latent. The ground for such an assumption is not apparent.

4. From the phylogenetic point of view, how far back must the atavism of scutes point? The whole matter of the phylogeny of turtles is obscure, but, from paleontological data, it is evident that the carapace of Thecophorous turtles acquired at quite a remote period very nearly the present form as regards arrangement of scutes and plates. Existing genera may be traced back to Eocene (*Thalassochelys*) and Upper Cretaceous periods (*Chelone*). More significant still are such fossil forms as *Plesiochelys solodurensis* Rüttimeyer, and *Platycheilus oberndorferi* Wagner, from the Upper Jura, with carapaces showing essentially the same plan of scutes and plates as is found in turtles of to-day, except that the dovetailing of neural and costal scutes is less noticeable. I should be slow to draw inferences from the variations in modern turtles regarding the types of periods more remote than the Upper Jura.

Finally, in the abnormalities we have to do not merely with *supernumerary scutes* but with many instances of turtles with less than the typical number of elements, which cases it would be difficult to explain primarily by reversion. If turtles may have *less* than the typical number (even to only *three scutes in each* dorsal series, in No. 35), otherwise than by reversion, why may not they have *more* in the same way?

#### SUMMARY OF PART III.

1. The experiments in incubation are suggestive of the possible significance of external conditions in the causing of abnormalities of scutes.

2. I can attribute to supernumerary scutes no value for deductions as to the phylogeny of turtles.

3. Whether or not atavism may have a remote or indirect connection with the abnormalities (for which I see no evidence) I do not regard individual scutes as atavisms.

4. Many of the abnormalities are clearly *not* atavisms.

#### Note.

As my conclusions in regard to the significance of abnormality of scutes is in opposition to that with which Newmann starts out, and may, therefore, seem inimical to his hypothesis, I think it proper to state that the value of Newmann's view of the evolutionary history of the carapace appears to me to be largely independent of the question of atavism since the more essential features were really based on comparative anatomy, not systematic atavism. He supposes fourteen primitive series of scutes; of the five not present in most turtles, only one was found in his *abnormalities* (interplastrals), while three were represented by *normal* series of certain species (interplastral and supramarginals, two) and another only by *normal* scutes of the tail of Chelydra. My observations might, it is true, show in abnormality representatives of the supramarginal series, but, just as well, they would give reason to infer primitive division of neurals and the primitive presence of a pair of *submarginal* series, neither of which inferences would be in harmony with his view. Hence the

number of series involved in his view seeks its basis, not in supposed atavisms, but in comparative normal anatomy.

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## EXPLANATION OF PLATES.

Figs. 1-13, plates I-VI, photographs of shells of diamond-back terrapin.

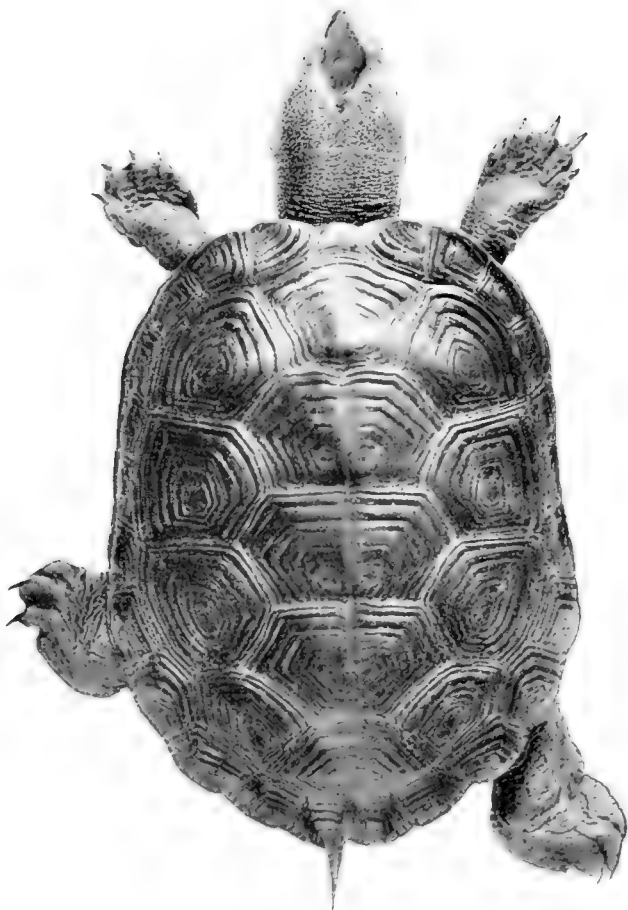
Figs. 14-54, plates VII-XI, sketches of plan of scutes of diamond-back terrapin; when only a portion of the carapace is shown, the drawing is from a field-sketch; Fig. 51 omitted; Figs. 53 and 54 from terrapin of Texas (see p. 31).

Figs. 55-104, plates XI-XIV, drawings of plan of scutes of embryos and new-born of the loggerhead sea-turtle.

*In the first eleven plates the figures in parentheses following the number of the illustration refer to the serial number of the specimen in tables I-V; in the succeeding plates (and in plate XI if preceded by the letter "T") the numbers refer to the specimens of Thalassochelys of tables VI-IX.*





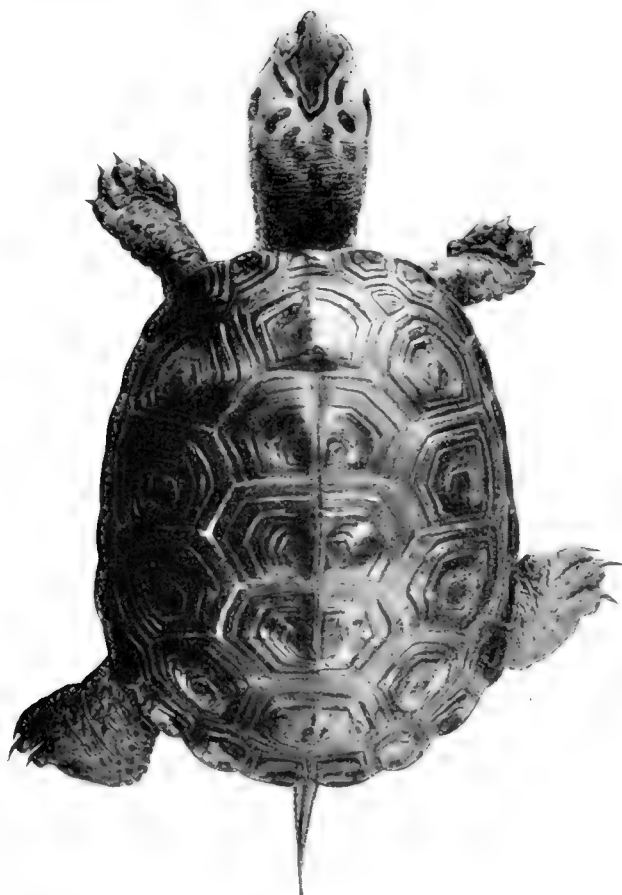


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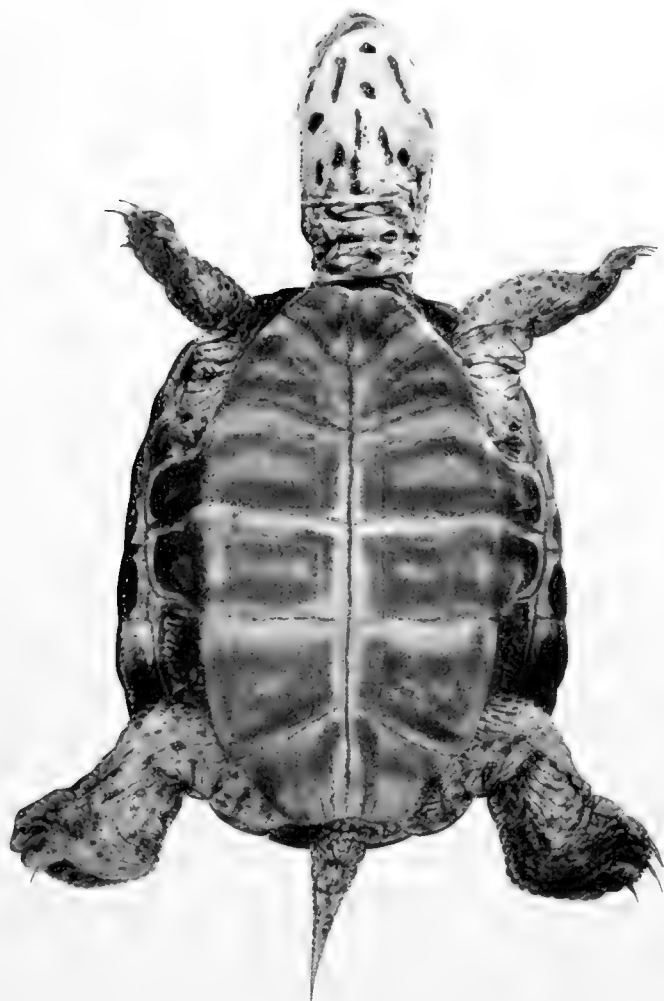


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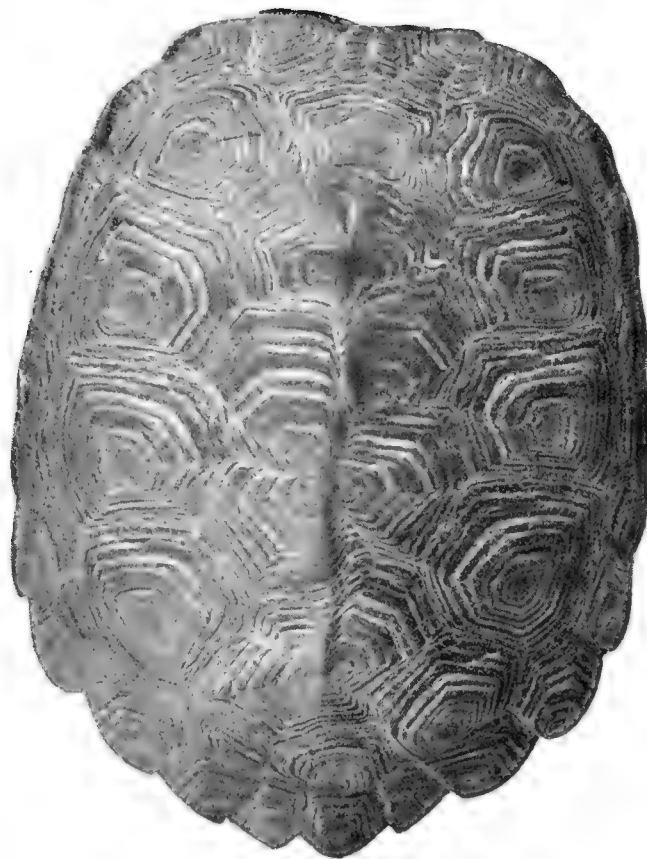


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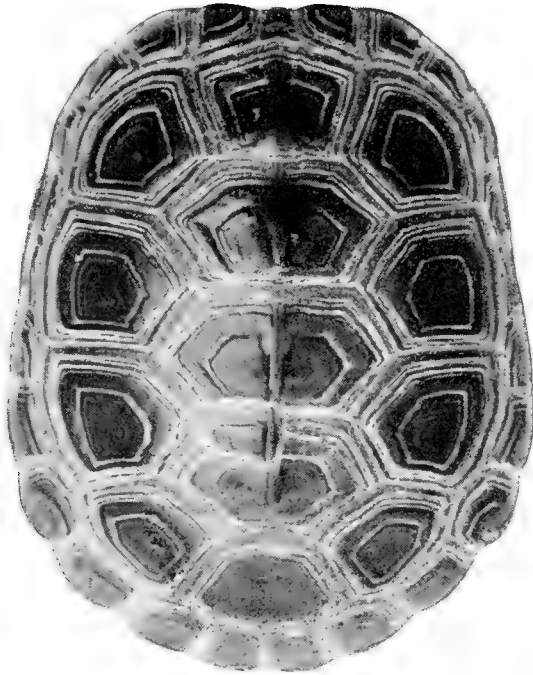


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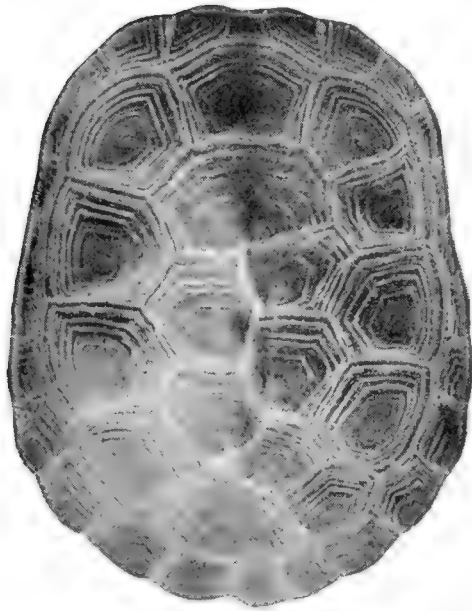


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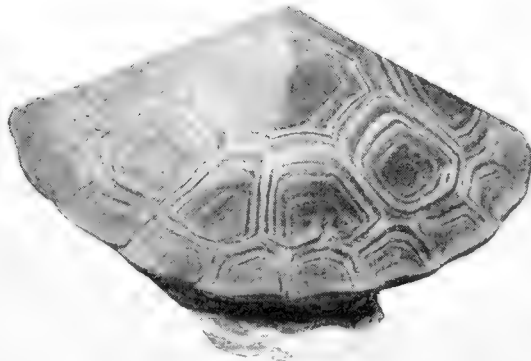




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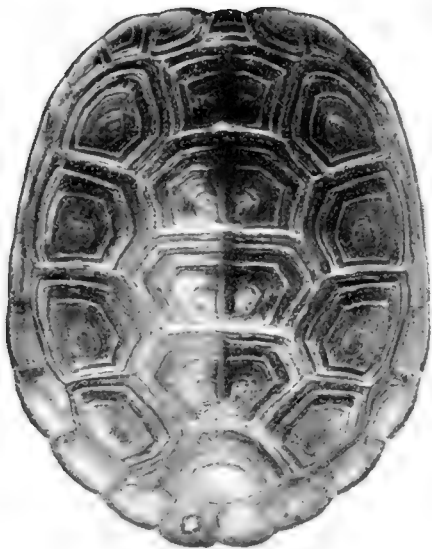
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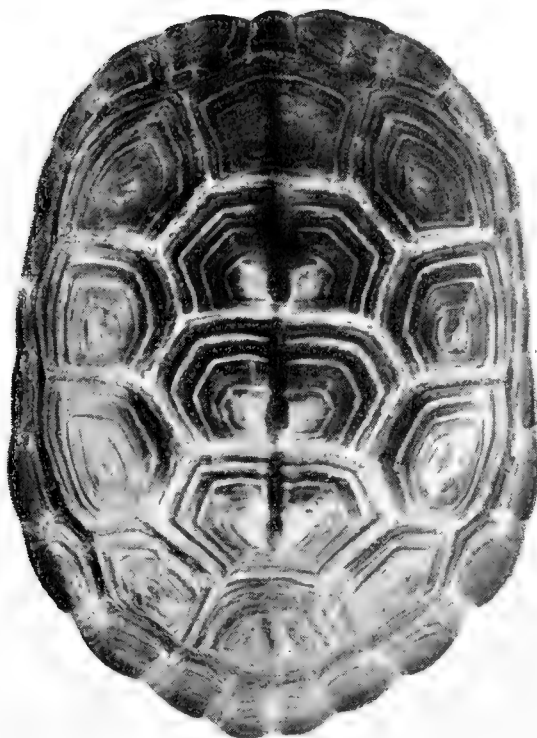


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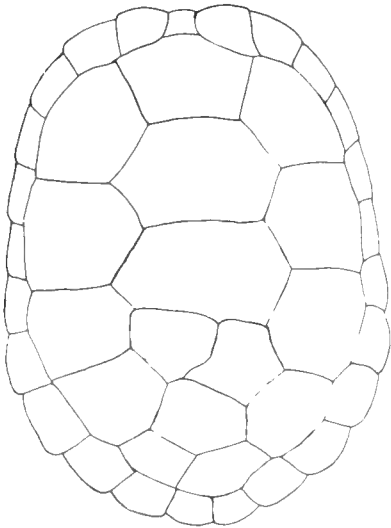


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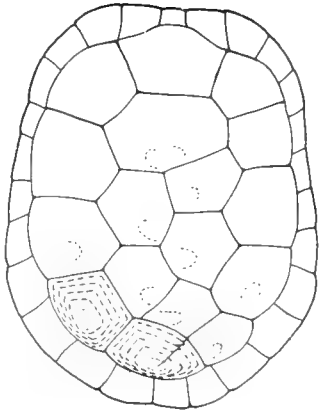


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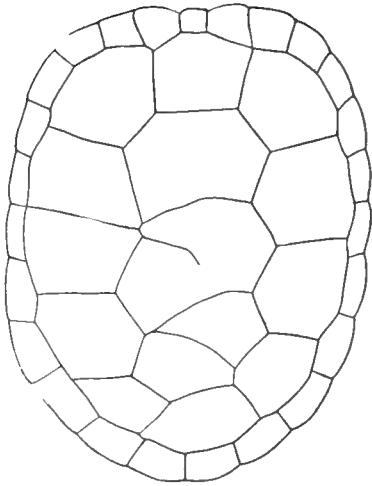




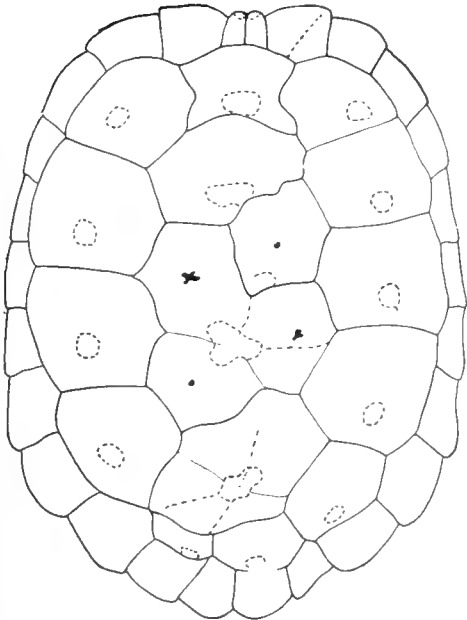
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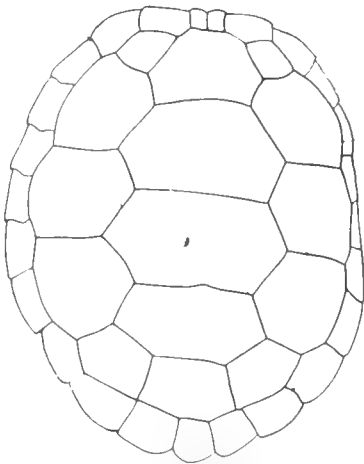
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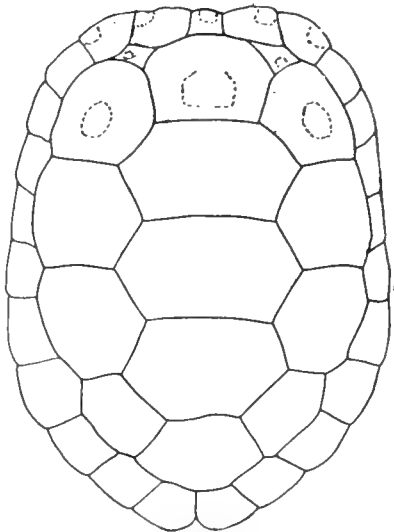
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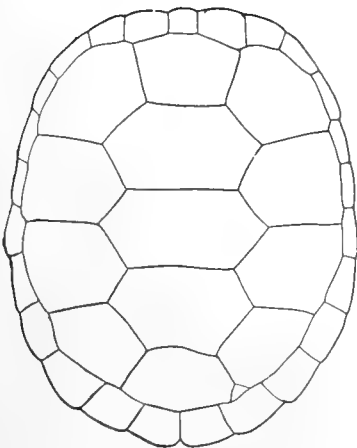
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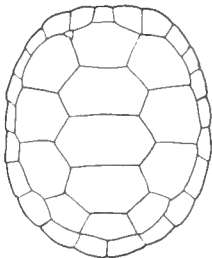
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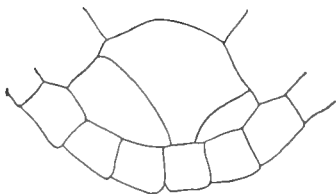
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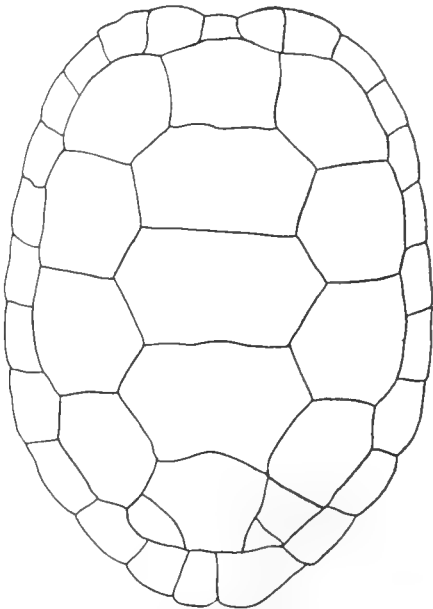
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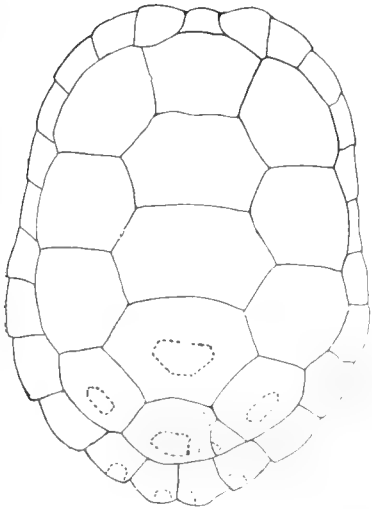


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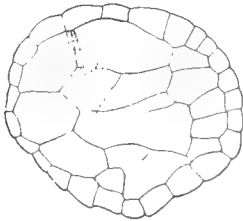


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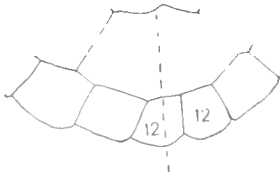




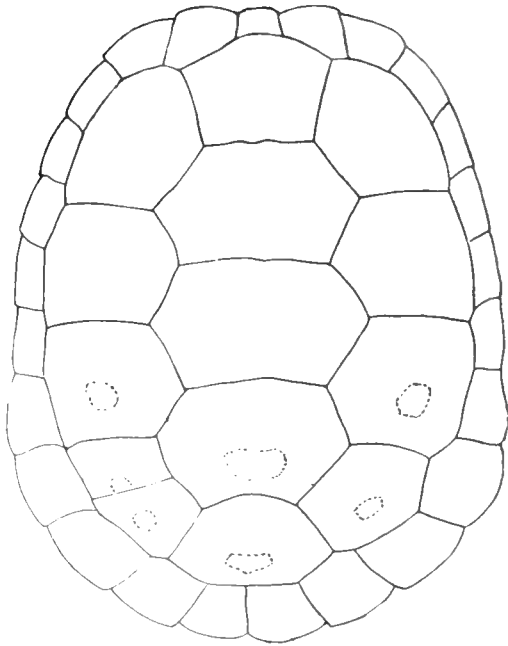
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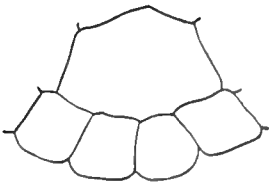
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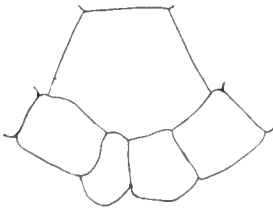
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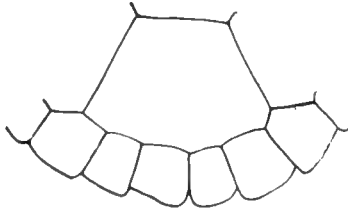
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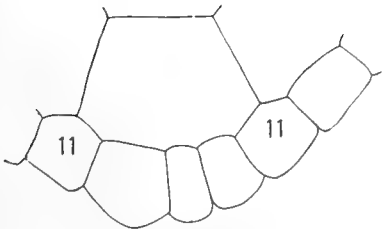
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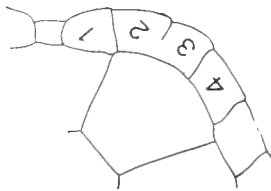
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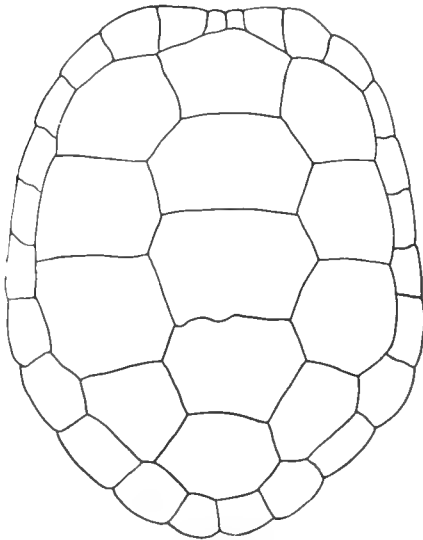
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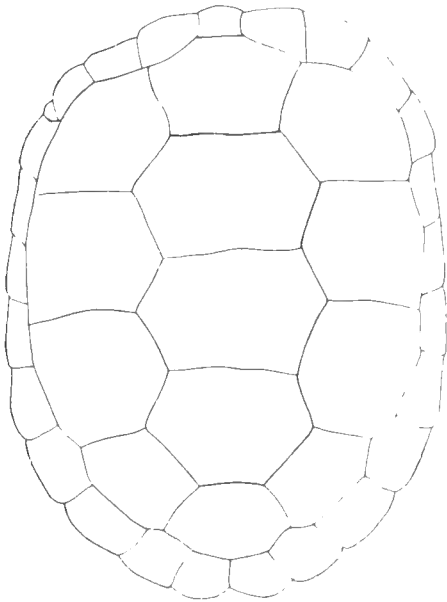


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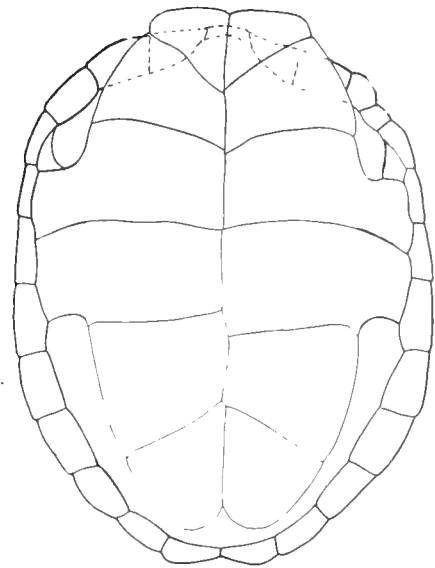




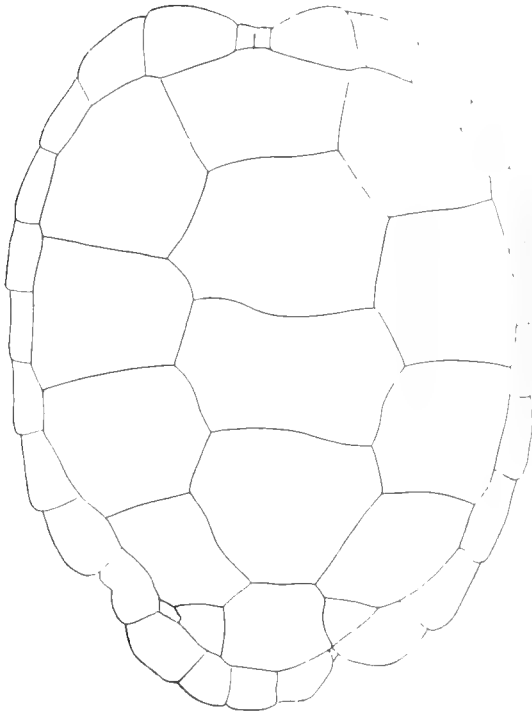
ROBERT E. COKER.



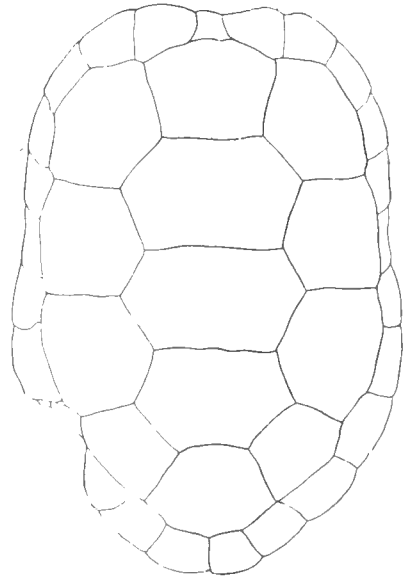
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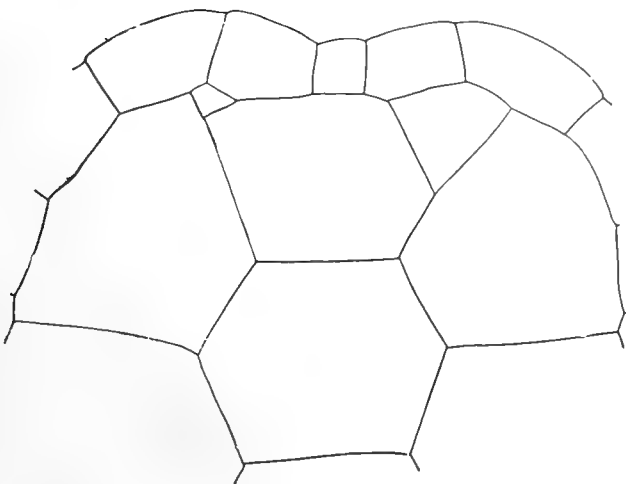
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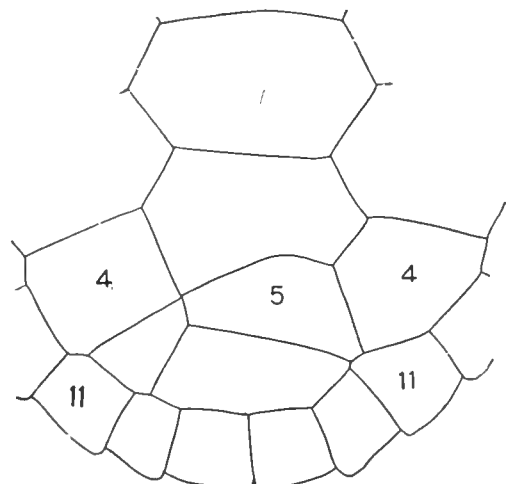
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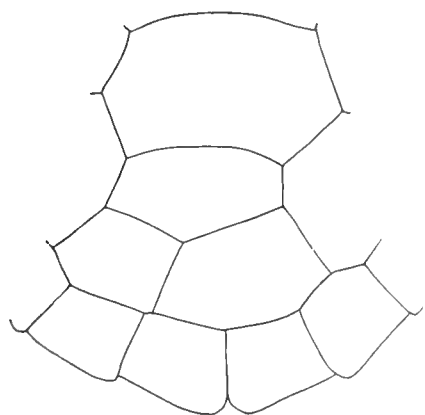


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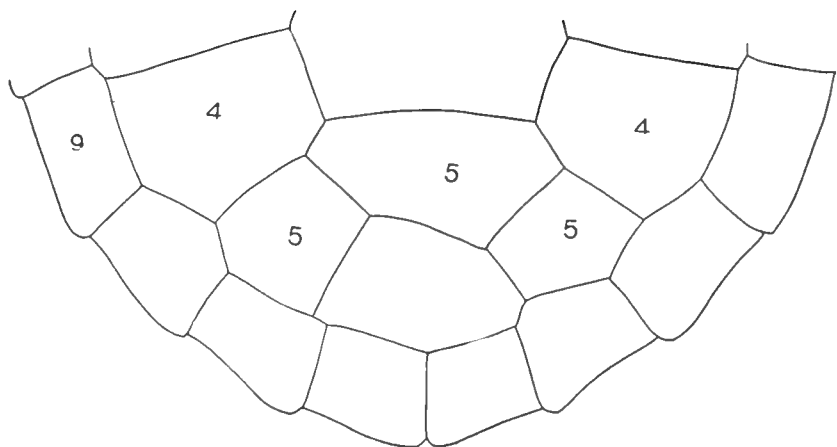


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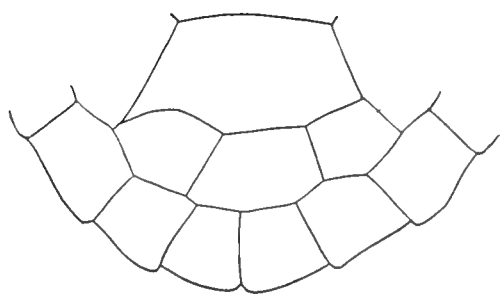




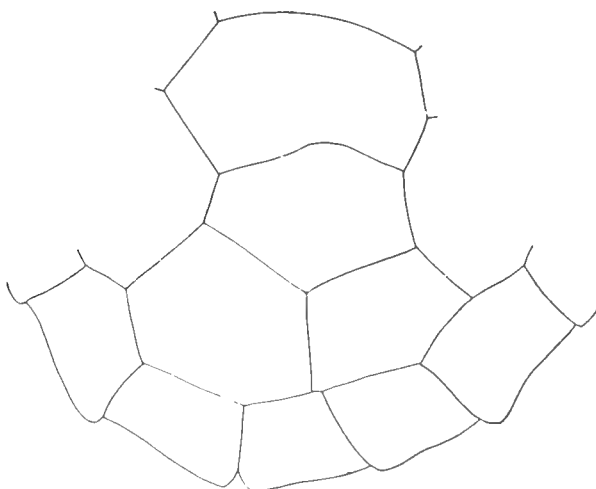
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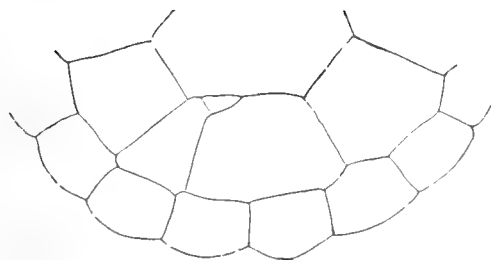
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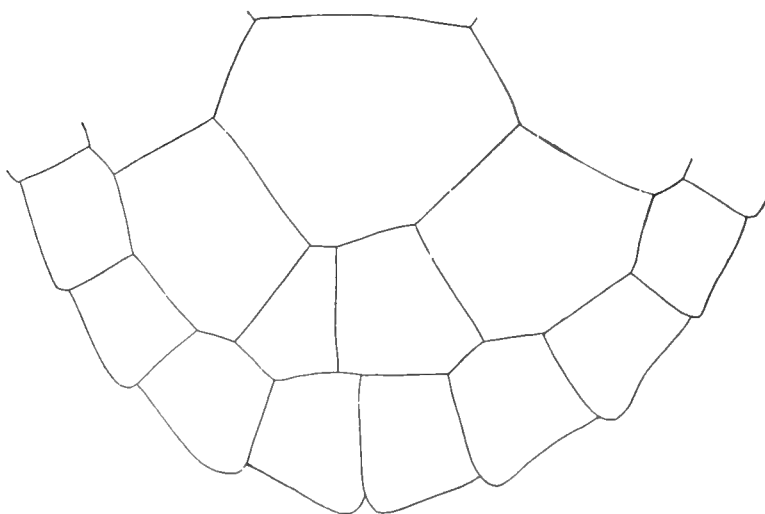
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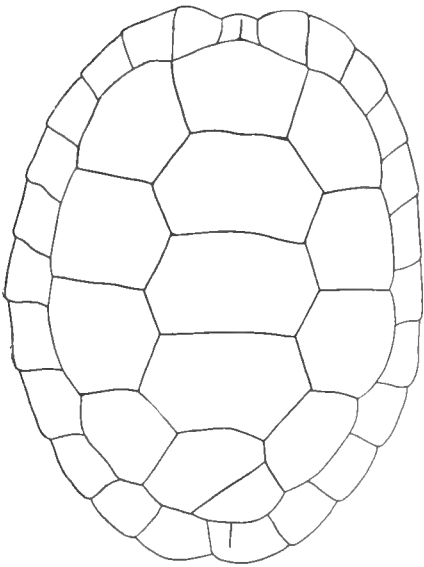


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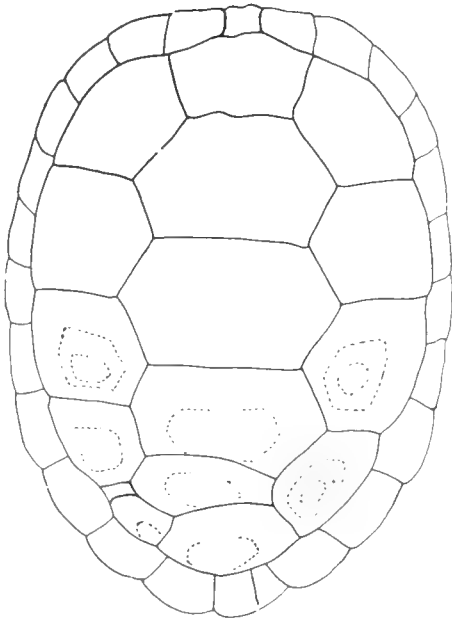


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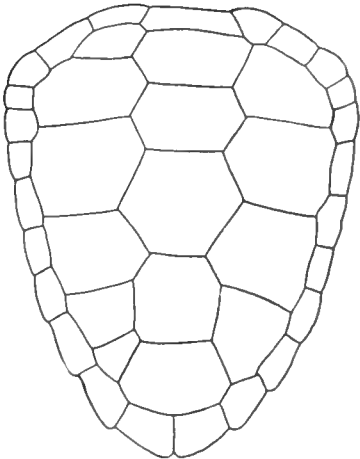




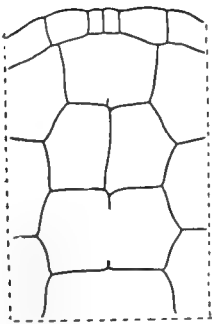
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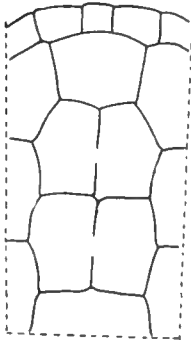
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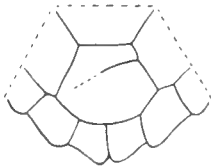
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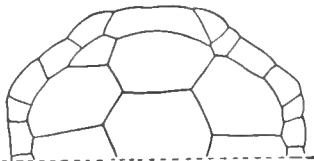
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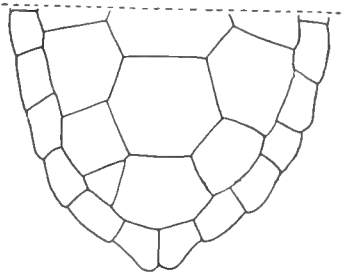
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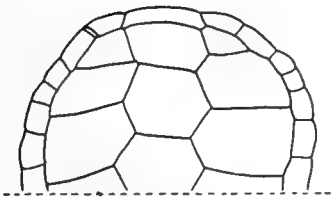
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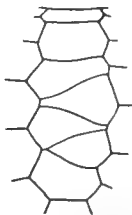
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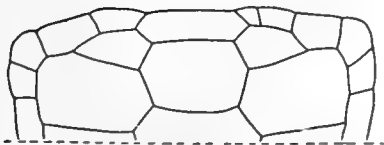
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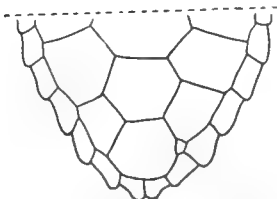
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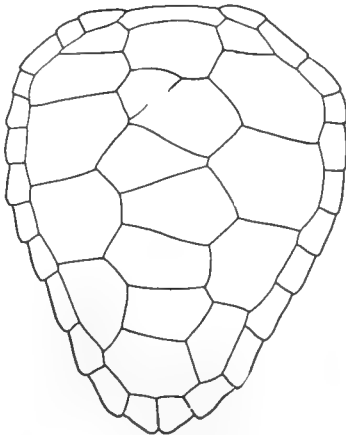
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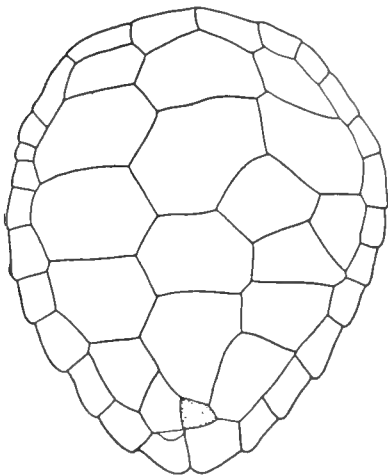


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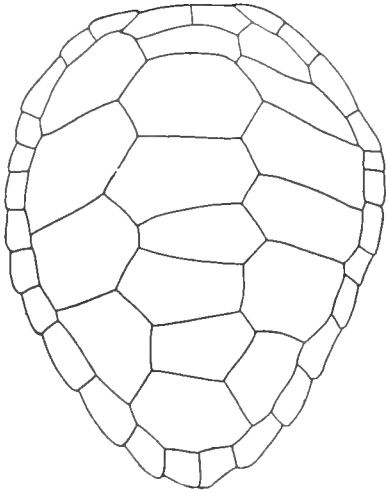


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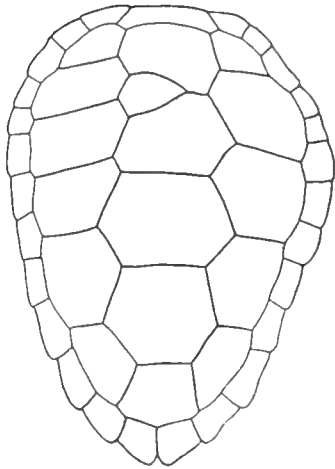




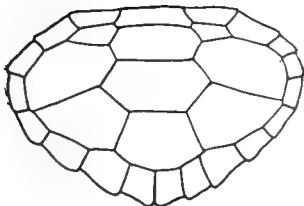
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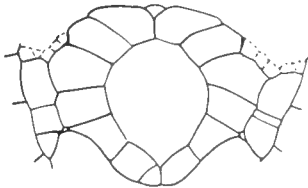
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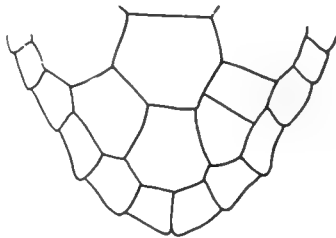
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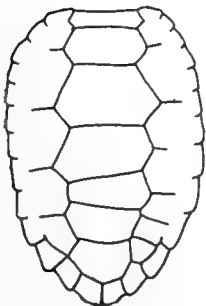
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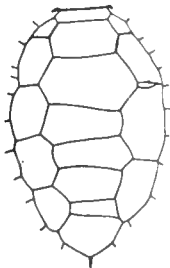
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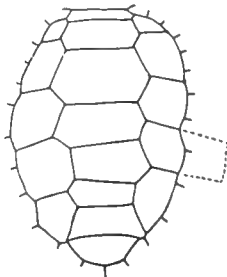
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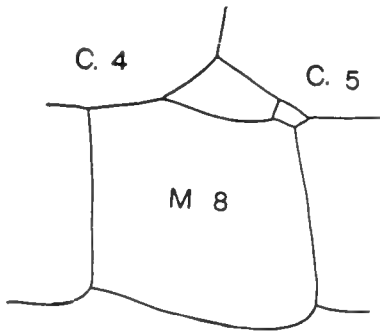
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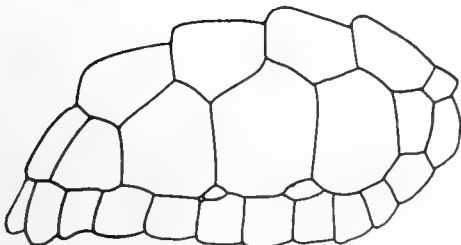
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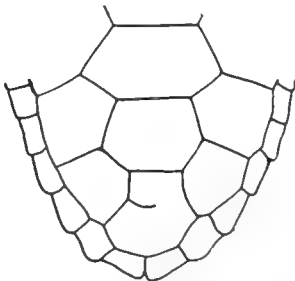
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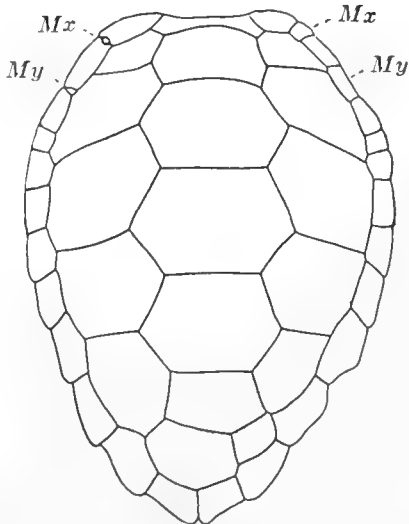
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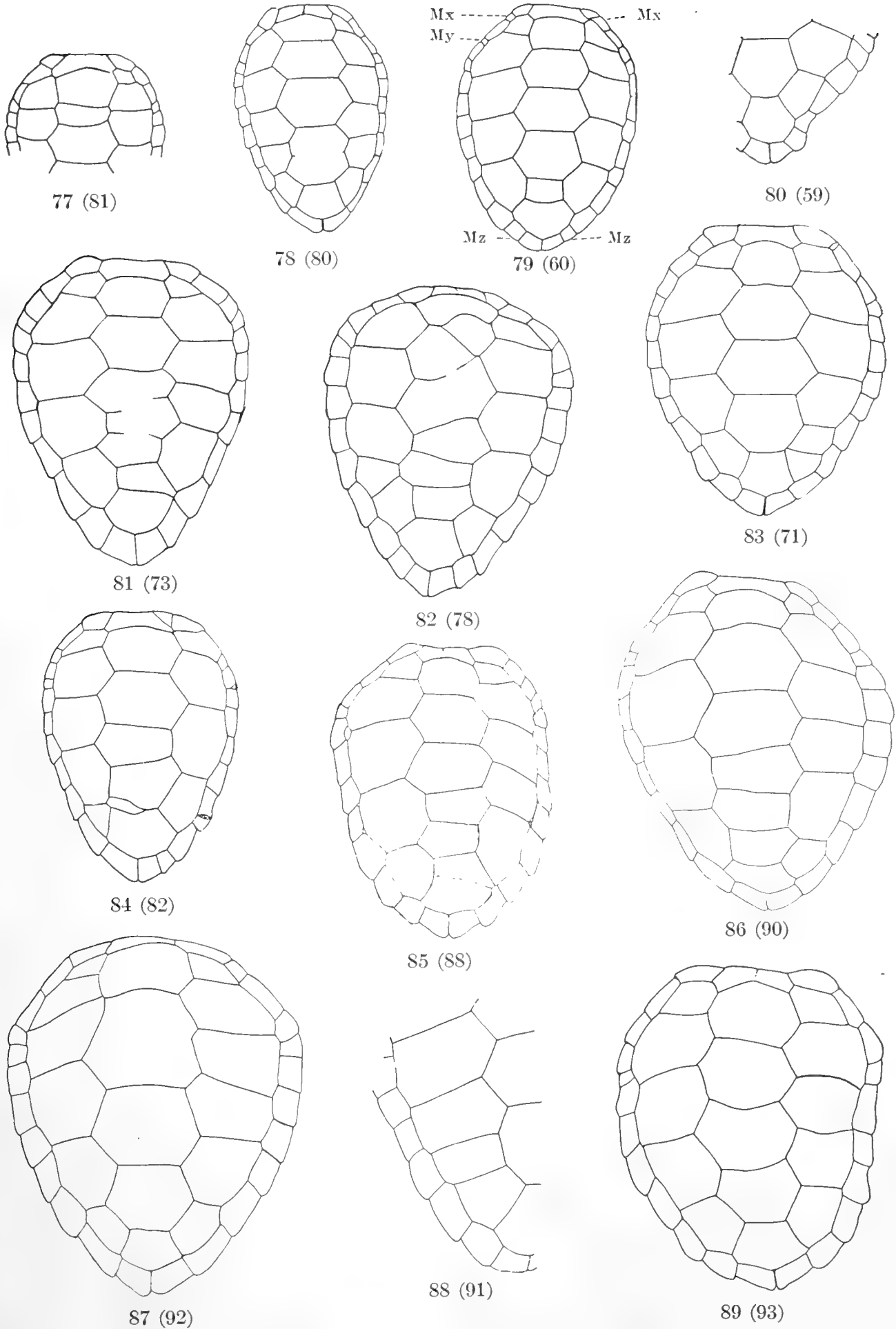
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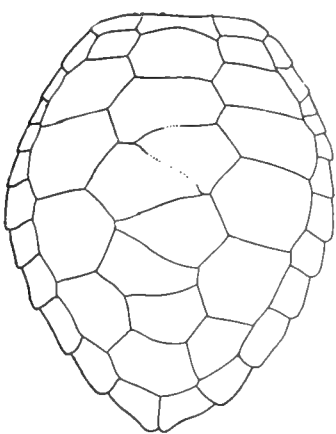
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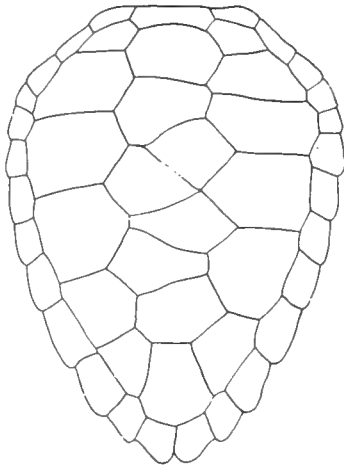




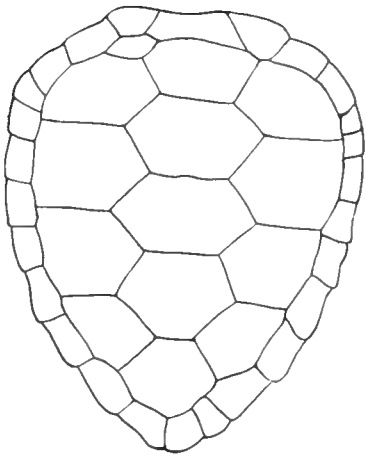




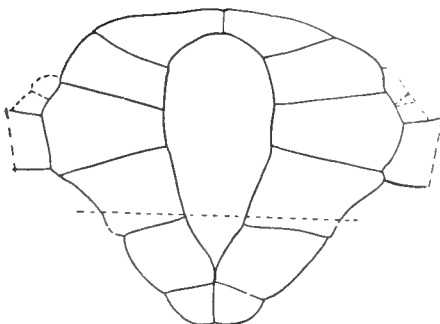
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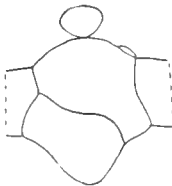
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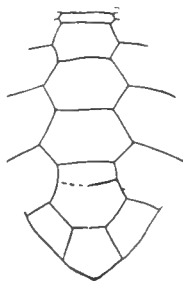
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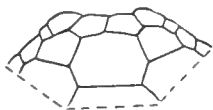
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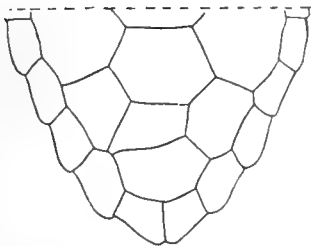
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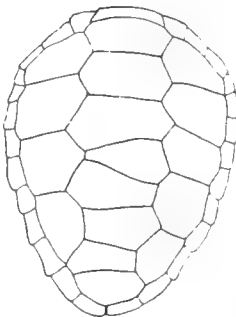
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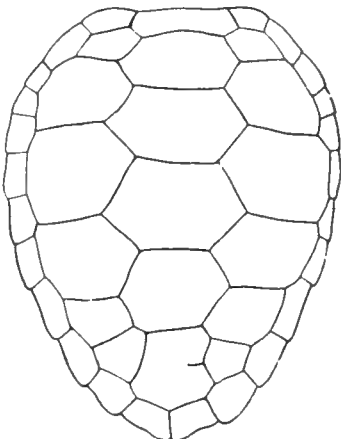
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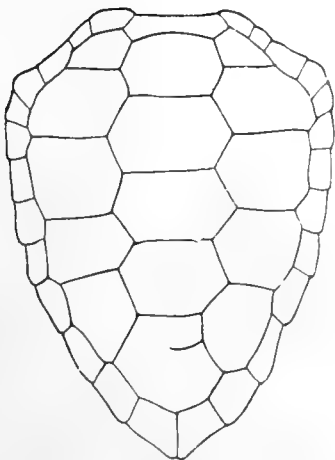
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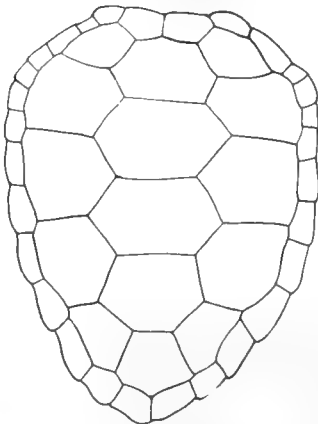
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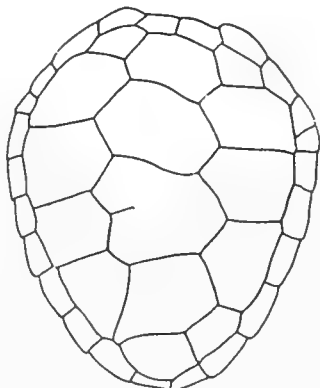
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# THE OSTEOLOGY AND MUTUAL RELATIONSHIPS OF THE FISHES BELONGING TO THE FAMILY SCOMBRIDÆ.

EDWIN CHAPIN STARKS.

This investigation of the osteology of the family Scombridæ was not undertaken to decide any question of obscure relationship of the groups within the family to each other, but to serve as a foundation for future work on the relationship of the many forms that have from time to time been placed in families supposed to be more or less closely related to the Scombridæ, and known collectively as the group Scombroidei—the mackerel-like fishes.<sup>1</sup>

Though the family Scombridæ probably does not contain the most primitive of the Scombroids it has served as a center around which the more or less aberrant forms have been arranged, and is consequently a convenient basis from which to work.

The family may be characterized as follows:

Bones all light and fibrous.

Supraoccipital crest formed anteriorly by frontals; usually extending to the ethmoid.

Supraoccipital not separating exoccipitals or epiotics, though more or less completely covering the epiotic suture on the surface of the skull, and sometimes the exoccipital suture.<sup>2</sup>

<sup>1</sup>This paper will be followed by others, each treating of a family, or of a few related families, of the Scombroid fishes. Only mutual relationships within the family will be discussed, though relationships between families will be touched upon when it seems advisable. This latter question, however, as well as the relationships of the group to other groups will be more fully discussed in a final paper.

<sup>2</sup>As this character can be seen only by bisecting the cranium it has scarcely been reported upon. When a bone on the surface of the cranium covers a suture between two other bones they are erroneously said to be separated by that bone. Of the few Percoids examined for this character none were found with the epiotics in contact with each other.

Exoccipitals (except in Scombrinæ) broadly meeting over basioccipital.

Prefrontals broadly united at the median line; each pierced for the passage of the olfactory nerve.

No auditory bulla apparent externally.

Temporal crests well developed, and reaching at least to above middle of eyes.

Myodome large and opening posteriorly to the exterior.

Opisthotic (except in Scombrinæ) interposed between the pterotic and exoccipital, and the lower limb of the posttemporal attached to its upper surface.

Basisphenoid present, and with a process descending to the parasphenoid.

Eye with a bony sclerotic case.

Suborbital ring incomplete, and without a sensory tube (except in Scombrinæ).

Nasal bones well developed.

Process of premaxillary short, heavy, and triangular, and abutting immovably against the ethmoid.

Maxillary with an auxiliary element on its upper edge.

Preopercle unarmed with spines, and all of the opercular elements fitting closely together to form a broad smooth plate.

Head of hyomandibular where it articulates with cranium divided into two parts.

Teeth of jaws fitting into alveoli.<sup>3</sup>

Four pairs of superior pharyngeals present; the third and fourth on each side sometimes joined (not ankylosed) to form a more or less complete single plate.

Three basibranchials present; the first remote from the hypohyal of the first arch, and hooked under the glossohyal.

Clavicle not developed much above pectoral fin, placed very oblique and sloping forward, beyond the hypocoracoid.

Actinosts four in number; short and broad; two and a half of them on the hypercoracoid.

<sup>3</sup>This character is appreciable only in the forms with moderate or large teeth.

Postclavicle in two parts.

Parapophyses never developed far anteriorly; when they appear they soon become attached to form hæmal arches and spines at about the middle of the abdominal cavity.

Ribs posteriorly at the tips of the abdominal hæmal spines, each pair with their bases in contact.

The epipleurals never borne by the ribs except in *Acanthocybium*.

Baseosts expanded at base of dorsal spines to form broad bony bucklers.

First interhæmal never much enlarged, nor strongly attached to the first hæmal spine.

United parapophyses in no way differentiated from hæmal arches, so that abdominal vertebræ are only distinguished from caudal vertebræ by the attachment of ribs.

Caudal rays deeply divided to receive hypural plate, which they usually completely cover.

Dorsal spines weak and flexible; dorsal and anal with some posterior rays detached to form finlets.

Caudal peduncle very slender; the caudal rays very divergent.

The principal sub-family and generic differences are indicated in the following key:

A. Pterotic not excluded from brain cavity by a deep pit behind prootic.  
No bony caudal keel except in *Sardinæ*.

B. The opisthotic situated on lower surface of cranium; not interposed between the exoccipitals and pterotic; the lower limb of posttemporal attached to its posterior edge. The myodome open behind in a small transverse slit. The ethmoid produced to a strong angle in front; nasals narrow and projecting far beyond them. No lateral caudal keel either bony or membranous. Suborbital ring complete with a sensory tube.  
SCOMBRINÆ.

1. Gill rakers moderately long; metapterygoid not supporting pterygoid.  
Scomber.

2. Gill rakers extremely long; metapterygoid assisting quadrate to support pterygoid.  
Rastrelliger.

BB. Opisthotic as much on superior as inferior surface of cranium, and interposed between the pterotic and exoccipital; lower limb of posttemporal attached to its superior surface. Suborbital ring incomplete, and without a sensory tube.



- C. Ethmoid concave in front. Nasals broad and attached for their full length to frontals and ethmoid. Myodome opening directly to exterior through a longitudinal foramen.
- D. Temporal crests reaching straight forward to near front of cranium. Caudal keel composed of membrane only.<sup>4</sup> The last two or three vertebræ normal in size.<sup>5</sup>
- E. Supraoccipital crest reaching to ethmoid; the full length of cranium concave on each side of it. Preorbital part of cranium not produced. The opening between alisphenoids to brain chamber wide. The vertebræ number 49. SCOMBEROMORINÆ. Scomberomorus.
- EE. Supraoccipital crest ending at front of eyes, anterior to which the broad preorbital part of cranium is transversely and evenly rounded and noticeably lengthened. The alisphenoids nearly divide the anterior opening to the brain case into two parts. The vertebræ number 66. ACANTHOCYBINÆ. Acanthocybium.
- DD. Temporal crests slanting obliquely to supraorbital rim. Caudal with a wide bony keel. The last two or three vertebræ abruptly decreased in length. All of which characters as in the Thunninæ, differing only as in division "A." SARDINÆ. Sarda.
- AA. Pterotic excluded from the brain chamber by a deep pit or infolding of the bone behind prootic. Caudal peduncle with a bony lateral keel.
- CC. Ethmoid produced to a medium angle in front; the nasals slender and much projecting beyond it. Myodome opening to a more or less specialized chamber in the parasphenoid. THUNNINÆ.
1. Inferior vertebral processes normal. Thunnus.
  2. Hæmal arches enormously developed and close to the centra. Euthynnus.
  3. Hæmal arches not enlarged but carried far away from the centra by a long bony pedicle. Auxis.

If we could eliminate the genus *Scomber* the family would be much more compact, as it stands farther from the other genera than they do from each other. The characters of *Scomber* may be for convenience here summed up a little more fully than in the foregoing key. It differs from all of the others in having the superior

<sup>4</sup>I am indebted to Mr. Barton A. Bean of the National Museum for investigating the condition of the caudal keel in *Acanthocybium*.

<sup>5</sup>This character has not been verified in *Acanthocybium*.

cranial crests peculiar; the opisthotic, and the attachment of the posttemporal to it normal; the suborbital ring complete and carrying a sensory tube; the auxiliary maxillary very small; the exoccipitals not meeting above the basioccipital, and with their condyles small and peculiarly placed, and in having neither a bony nor a membranous caudal keel.

Rastrelliger, Jordan and Starks, has departed but slightly from Scomber, differing chiefly in the way in which the metapterygoid assists the quadrate in supporting the pterygoid; in the high knife-like ridge formed by the basibranchials; and in the great development in the length of the gill-rakers. It is otherwise as in Scomber.

Certain characters indicate that *Scomberomorus* has come more directly from the Scombrinae than have any other of the genera here considered, though its evident alliance with *Acanthocybium*—possibly the most aberrant of its family—shows how far it has departed from the Scomber type. Consequently the fact that *Scomberomorus* is here most closely approximated to Scomber does not necessarily mean that it is the most closely related to that genus, but that its descent is more directly traced. It has no bony caudal keel, but a membranous one shows a development in the direction of one; its cranial crests are directed straight forward, though they are not interrupted as in Scomber; its auxiliary maxillary is small; and its last two or three vertebrae are normal, or not abruptly decreased in length.

*Acanthocybium* naturally comes next to *Scomberomorus*, though as is intimated above it certainly does not deserve a position so close to Scomber. It shows, as was long ago pointed out, an apparent divergence towards the sword fishes.

The exact position of *Sarda* is a little obscure. It has the concave ethmoid and non-projecting nasals of *Scomberomorus* and *Acanthocybium*, but has the cranial crests arranged almost identically as in the Thunninae. On the lower surface of the cranium is a slight depression showing a development towards the deep pit of the Thunninae and the caudal peduncle has a lateral keel. Consequently it must have sprung from somewhere between the Scomberominae and the Thunninae to have such marked characters of both groups. It

shows, however, a much closer alliance with the latter sub-family than with the former.

Thunnus (the genus *Germo* is not here recognized as distinct) is plainly close to Auxis and Euthynnus, as is shown by the exclusion of the pterotic from the brain cavity, and in nearly all characters but the condition of the infra-vertebral processes. It is rather astonishing to find genera running so close together as these three do, and yet differing so radically in the condition of the hæmal arches.

Lütken<sup>6</sup> calls the peculiar condition of the inferior vertebral processes in Auxis a modification of the condition of these processes in Euthynnus. This can scarcely be so, as the modification, though as extraordinary in Auxis as in Euthynnus, is of a different character. It is difficult to imagine either condition as being a modification of the other. It seems probable that these two genera left their parent stem at about the same place, or in other words that they are both a modification of some similar condition in the common ancestor from which the development has been divergent.

In Euthynnus the inferior vertebral foramina and the hæmal arches are enormously developed, the latter springing almost directly from the centra of the vertebræ, while both the postero and antero-zygopophyses equally form long slender processes between the arches.

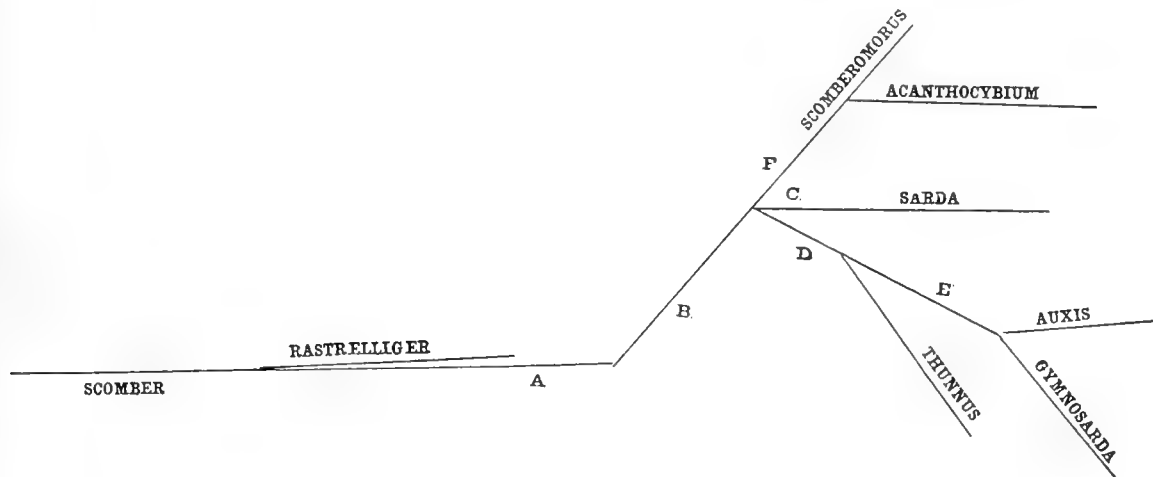
In Auxis neither the hæmal arches nor parapophyses are enlarged, and they are borne far away from the body of the vertebræ each by a solid bony pedicle formed (at least in part) by the antero-zygopophysis; the postero-zygopophysis taking no part in this formation.

So to consider the condition of Auxis as a modification of that of Euthynnus we should have to eliminate the postero-zygopophyses together with the laminæ of bone that incloses the inferior foramen behind each arch, join the antero-zygopophyses and the bone surrounding the front of the inferior foramen into a solid pedicle, and restrict the great arch to a small opening at the distal end of the pedicle.

The foregoing may be summed up by the following diagram showing the supposed origin of the genera.

<sup>6</sup>*Spolia Atlantica*, p. 596, 1880.

## DIAGRAM.



- A. The characters of Scomber.
- B. The acquirement of the interposed opisthotics.
- C. The Thunnus type of cranial crests, and the inferior cranial pit indicated, with the Scomberomorus type of ethmoid and nasals.
- D. The inferior cranial pit excluding the pterotic from the brain cavity, and the condition of the ethmoid and nasals of Scomber.
- E. The condition of the infra-vertebral processes from which Auxis and Euthynnus have diverged.
- F. The Scomberomorus type of cranial crests, elongate form, concave ethmoid, and nonprojecting nasals.

In the following pages the osteology of the genera is described in greater detail.

SCOMBER.<sup>7</sup>

A specimen of *Scomber japonica*, Houttuyn, 11 inches in length, from the Canary Islands, and a skull of a slightly larger specimen of the same species from Peru.

The supraoccipital crest is developed backwards in a long spatulate process, which is free from the exoccipitals below. On top of the cranium it is only slightly developed, and is carried forward by the frontals to opposite the beginning of the posterior third of the orbital

<sup>7</sup>For descriptions in detail of the osteology of *Scomber* see the beautifully illustrated work by Edward Phelps Allis, Jr., entitled "The Skull, and the Cranial and First Spinal Muscles and Nerves in *Scomber scomber*." *Jour. Morph.*, Vol. xvii, 1903.

cavity, or coterminous with the temporal crests. From the front of the supraoccipital a broad low rounded ridge runs obliquely across the frontal outward and forward toward the middle of the orbital cavity where it becomes lower and broader and merges into the general level of the frontal. Against this ridge the pterotic and temporal crests end a short distance from the orbital rim. A thin high auxiliary crest is developed between the anterior ends of the temporal and pterotic crests. The myodome is large, but posteriorly its opening is closed all but a small transverse slit by the parasphenoid.

The supraoccipital widely separates the parietals and is developed a little anterior to them. Posteriorly it covers the suture between the extreme upper ends of the exoccipitals, but it nowhere separates them. The exoccipitals do not meet over the basioccipital at the mouth of the long tunnel-like foramen magnum, though some distance in the foramen they are in contact for a short distance. The vertebral condyles of the exoccipitals are very small. They are anterior to the basioccipital condyle instead of overhanging it as usual, and they slope outward and forward away from the median line, rather than outward and backward or toward the median line. The pterotic ends posteriorly in a sharp spine and there is no deep pit between it and the prootic and sphenotic. There is no pit on the lower part of the cranium behind the prootic, and the pterotic is not excluded from the brain cavity. The opisthotic is as in the majority of spiny-rayed fishes: covering the suture between the pterotic and exoccipital, but not at all separating them. It is wholly on the ventral surface of the skull, and the lower limb of the posttemporal is attached to its posterior edge. The lateral process of the parasphenoid does not reach to the upper edge of the prootic. The vomer is broad and thick and on its outer anterior edge is an oblique facet, which fits snugly against the inner surface of the maxillary. The vomer and palatine bear small teeth.

The prefrontals broadly meet at the median line behind the ethmoid; they are pierced by the olfactory foramina. There is an articular facet on the posterior end of each, and another on the anterior end of the ethmoid for the attachment of the palatine. The ethmoid is but little posterior to the front of the vomer, and it is broadly rounded

anteriorly; the short premaxillary processes scarcely reach to it. A basisphenoid is present and is separated from the roof of the myodome as usual; it has a process descending to the parasphenoid. The alisphenoids do not meet each other.

A well developed preorbital is present and the suborbital ring is complete, consisting of thin plates along the anterior edge of which runs a small sensory tube. There is no suborbital shelf, and the eye has a bony sclerotic case as do all the other members of the family. The nasals are attached to the sides of the frontals and project far forward beyond the ethmoid.

The head of the hyomandibular, by which it is attached to the cranium, is divided into two parts, a round knob in front and an elongate portion behind, well separated from each other. The metapterygoid is channeled on its posterior edge to receive the edge of the hyomandibular, and behind the hyomandibular it sends a long triangular process backward half way across the preopercle. It has a large articular facet on its posterior edge to support the opercle. The symplectic is long and slender, somewhat broadened behind the metapterygoid, and extending behind the quadrate in a channel. The opercle bones are wide and fit smoothly together without ridges; the preopercle in particular is broad. The wedge-shaped process of the articular does not nearly fill the usual deep notch of the dentary, thus leaving a considerable space between these two bones along the upper edge. A well developed angular is present. The premaxillary and dentary teeth are small and set in alveoli. On the inner anterior edge of the maxillary is a large articular facet for attachment to the side of the vomer. On the posterior upper edge is a small auxiliary maxillary. The maxillaries are not in contact with each other anteriorly. The premaxillary processes are heavy, short, and triangular, and they do not project backward to reach the ethmoid.

The hypohyals are very large and are paired on each side; a wide flat glossohyal is present; the broad urohyal has scarcely any lateral wings developed along its lower edge. Four branchiostegal rays are attached to the ceratohyal and three to the epihyal; the anterior ones are attached to the lower edge of the arch, but they creep up to the outer surface posteriorly. A short interhyal is present.

Three bony basibranchials are present; that of the fourth arch is cartilaginous. The second and third arches join the third basibranchial, the first arch joins the second, and the first basibranchial is wholly in front of the arches, and projects forward under the glossohyal. As usual there is no hypobranchial to the fourth arch. The inferior pharyngeals are wide and are thickly covered with long brush-like teeth. There are four superior pharyngeals present on each side. The third and fourth together form a single elongate plate though they are not anchylosed to each other. The second is small and narrow and lies beside the anterior part of the third rather than in front of it. The first is as usual toothless; the others are covered with teeth similar to those on the lower pharyngeal, but smaller.

The clavicle is a long evenly curved bone with a broad wing projecting backward over the pectoral for the support of the postclavicle. The hypercoracoid foramen is large and just below the center of the bone. The hypocoracoid is long and slender and arches away from the clavicle to rejoin it again a considerable distance above its point. From the upper edge of the hypocoracoid a wing is developed backwards to the tip of the lower actinost. The four actinosts are broad and short with a small pore between each pair; the third one from the top is supported equally by the coracoid elements. The postclavicle is in two parts, the upper broad and thin, the lower broad above but tapering to a long point downward. A short wide supraclavicle is present. The posttemporal is forked; the upper limb lies broadly over the epiotic extending slightly onto the supraoccipital; the lower limb is attached to the posterior edge of the opisthotic as in the majority of fishes.

The pelvic girdle is rather complex in shape, consisting of a thin horizontal plate, which meets its fellow of the opposite side at the median line. From the horizontal plate is developed downward a subvertical plate. From the union of the opposite sides of the girdle a pair of long thin processes are developed forward, and a pair of spine-like processes are developed backward between and above the ventral fins.

There are 15 abdominal vertebræ, and 15 caudal vertebræ, or a

total of 31 with the hypural. The parapophyses are not developed anteriorly. On the eleventh abdominal vertebra they first appear, but are here joined to each other to form a hæmal arch. Anterior to this on two or three vertebræ the edges of the socket into which the rib fits is slightly produced into a crater-like rim, but no developed process is present. On the first three hæmal arches no spine is present, but on the arch of the last abdominal vertebra the spine is long and similar to those on the anterior caudal vertebræ. Consequently aside from the fact that the abdominal vertebræ bear ribs, and the first caudal vertebræ bear the anal fin there is nothing to differentiate the abdominal vertebræ from the caudal. The inferior foramen is present in the base of the hæmal spine of the last abdominal vertebra, and in most of the caudal vertebræ. The last two or three vertebræ are not abruptly shortened. On the anterior caudal and posterior abdominal vertebræ each antero-zygopophysis reaches forward and forms a semi-inclosed space behind and below each inferior vertebral foramen. The first two vertebræ bear epipleurals only; the first ribs being on the third vertebra. The ribs are flattened and are directed backward so that they lie close to each other. When the hæmal arches develop, the ribs drop down to their tips and the bases of each pair of ribs are thus brought in contact. The epipleurals are attached directly to the vertebræ, and anteriorly their bases are in contact with those of the ribs, but posteriorly they hold their position at the bases of the hæmal arches, while the ribs drop down to the hæmal spines. The epipleurals are present back to opposite the posterior part of the anal fin. The interneurals of the spinous dorsal are much expanded at the upper end, but the baseosts, one of which is in front of each spine, are so broad they extend laterally far over the interneurals, and form a bony buckler that is visible under the skin of the undissected specimen. A long baseost extends between each of the finlets behind the dorsal and the anal fin. The first interhæmal is not enlarged. The caudal rays are deeply divided and so fit over the hypural plate that they cover it more than half from sight.

The elements not mentioned here are as they normally are in the great majority of spiny-rayed fishes.



## RASTRELLIGER.

A specimen of *Rastrelliger branchysomus*, Jordan and Starks, 12 inches in length, from the Fiji Islands.

The cranium is less depressed than in *Scomber*, though it does not differ in the crests and ridges of the cranium from that genus. The epiotics appear to meet very broadly posteriorly, but close examination reveals a slender spur from the supraoccipital extending down between them to the exoccipital suture. The top of the cranium in front of the oblique ridge that runs from the supraoccipital to the supraorbital rim is finely sculptured and thickened by a network of fine ridges where in *Scomber* the bone is smooth. The foramen magnum forms a long tunnel of the exoccipitals as in *Scomber*, and the condition of the exoccipitals over the basioccipital and their condyles is the same.

The mandible and maxillary elements are much weaker than in *Scomber*. The premaxillary is a long slender bone from which the maxillary arches widely away, being attached to it only at each end; the auxiliary maxillary is small. The most striking difference between this genus and *Scomber* lies in the arrangement of the lateral bones of the skull and the basibranchials. The pterygoid normally (as in *Scomber*) is attached along the anterior edge of the quadrate, at the upper end of which it bends at an angle forward to support the palatine; the metapterygoid is behind and a little above the quadrate. In *Rastrelliger* the metapterygoid is above and somewhat in front of the quadrate, and the pterygoid borders the entire front of both the quadrate and metapterygoid turning at an angle at the upper edge of the latter.

The basibranchials form a high, sharp, knife-like ridge, while the hypobranchials are deep and compressed and help to elevate the basibranchials still higher. The second and third superior pharyngeals are joined into a single plate a little more firmly and completely than in *Scomber*. The branchial arches are crowded backwards against and between the shoulder girdles; and in fact all of the bones of the head give the impression of having been drawn downward and backward and compressed.

There are 14 abdominal vertebræ and 16 caudal, or a total of 31

with the hypural. The vertebral elements are arranged as in Scomber, as are the other elements with the above exceptions.

## SCOMBEROMORUS.

A specimen of *Scomberomorus sierra*, Jordan and Starks, 24 inches in length, from Mazatlan, Mexico, and the head of a specimen of *S. maculatus* (Mitchill), 18 inches in length, from Chesapeake Bay.

This genus differs from Scomber in having the supraoccipital crest carried forward by the frontals to the ethmoid, and in having the cranium deeply concave for its full length on each side of the crest. The temporal crests are directed straight forward as in Scomber, but they are not interrupted above the eyes by a transverse ridge. They nearly reach to the ethmoid, and anteriorly between them and the pterotic crests are developing small auxiliary crests as in Scomber, but situated farther forward. The pterotic crest extends forward to above the middle of the eye. The myodome opens posteriorly through a wide longitudinal foramen.

The supraoccipital crest extends down over the exoccipital suture more broadly than in any other genus except *Acanthocybium*, though it is not at all interposed between them. The exoccipitals broadly meet for their full length above the basioccipital. Their vertebral condyles are large and slope back over the basioccipital as usual. The pterygoid does not end in a sharp spine posteriorly. The ethmoid is forked or concave in front to receive the premaxillary processes. The opisthotic is interposed between the exoccipital and pterotic, so that as much of it is on the superior surface of the cranium as on the inferior. The lower limb of the posttemporal is attached directly, or without the intervention of a ligament, to its superior surface rather than to its posterior edge.

The suborbital ring is incomplete; the preorbital is well developed and there are two small suborbital plates, the second developed as a small thin suborbital shelf. The rest of the ring is made up of the thick scales that cover the cheek, which are slightly turned inward at the border of the eye; they carry no sensory tube. A small thin Y-shaped supratemporal bone is present bearing a branched sensory tube just under the skin. The nasals are wide bones attached for their full length to the side of the frontal and ethmoid, and do not project at all beyond the latter.

There is no process sent backward from the metapterygoid across the inner surface of the hyomandibular. There is no space left between the articular and dentary. The maxillary bears a small auxiliary maxillary as in *Scomber*. The short premaxillary processes project into the concavity in the front of the ethmoid. The teeth in the dentary and premaxillaries are large and laterally flattened; the vomer and palatine bear small granular teeth.

The third and fourth superior pharyngeals are not nearly so closely attached to form a single plate as in *Scomber*, and the teeth on both the superior and inferior pharyngeals are smaller, stiffer, and less brush-like. The hypercoracoid foramen is large and through the center of the bone. The pelvic girdle is more slender and the vertical plate is but little developed.

The vertebræ number 19 abdominal, 29 caudal, or a total of 49 with the hypural. The condition of the parapophyses, zygopophyses, ribs, epipleurals, and interspinous bones is similar to those of *Scomber*. The hæmal and neural processes are more slender, fragile, and fibrous than in any other genus of the Scombridæ. The caudal rays lap over the hypural plate even farther than in *Scomber*, or until the bases of the opposite rays meet on the median line and hide the plate almost completely. The urostyle of the hypural is better developed.

Aside from these characters *Scomberomorus* is as described for *Scomber*.

#### ACANTHOCYBIUM.

A head of *Acanthocybium solandri*, Cuvier and Valenciennes, including the upper elements of the shoulder girdle and the first three vertebræ, from the Hawaiian Islands.

In this genus the cranial crests extend straight forward as in *Scomberomorus*, not obliquely towards the suborbital margin as in the *Thunninæ* and *Sardinæ*; there is no auxiliary crest as in *Scomberomorus*. Both the temporal and supraoccipital crests stop, however, at the front of the eye, and the top of the cranium anterior to them is evenly rounded transversely unlike any other genus of its family. The supraoccipital covers the exoccipital suture rather broadly as in *Scomberomorus*. The preorbital part of the cranium is noticeably

produced. In *Scomberomorus* the brain chamber is open widely between the alisphenoids, while in this genus the alisphenoids nearly meet at their middle and almost divide the opening into two parts. The vomer bears a patch of small teeth.

The nasals and suborbital ring are as in *Scomberomorus*, but the sclerotic case is thicker and denser than in any other genus. The teeth on the jaws are flat and saw-tooth-like with very sharp finely serrate edges. The anterior part of the premaxillaries project very much in front of the maxillaries to form a sharp beak, though only to a greater degree than in *Scomberomorus*; the auxiliary maxillary is well developed.

The lateral head bones including the hyoid and branchial elements exhibit no departure from the other genera.

The first two vertebræ bear epipleurals only, the first rib being on the third vertebra. This rib bears on its side, some distance from its base, an epipleural (the succeeding vertebræ are missing). In this respect *Acanthocybium* differs from all of the other genera, as in the others all of the epipleurals are on the centra. The vertebræ are said to number  $32 + 34 = 66$ .

#### SARDA.

A specimen of *Sarda chilensis*, Cuvier and Valenciennes, 30 inches in length, from Puget Sound.

The cranium is broad and depressed with moderately high thin crests. The supraoccipital crest is carried forward by the frontals to the ethmoid. The temporal crest bends outward from the epiotic and reaches the supraorbital margin above the middle of the orbit. The pterotic crest forms the posterior lateral margin of the cranium. All of the crests are nearly identical with those of the *Thunninæ*. The supraoccipital crest dips down a little farther to the exoccipitals than in *Thunnus*, but not so much as in *Scomberomorus*. It does not at all separate the exoccipitals and barely covers the suture between the posterior end of the epiotics. There is a very slight indication of the pit on the lower surface of the cranium that is so pronounced in the subfamily *Thunninæ*, but it in no degree excludes the pterotic from the brain cavity. There are no openings into the brain cavity at the end of the frontals. The myodome opens directly

to the exterior posteriorly through an elongate foramen, at the anterior end of which is a slight cavity in the parasphenoid indicating the chamber that is developed at this place in the Thunninæ.

The exoccipitals meet broadly above the basioccipital and have very large condyles, which slope normally over the basioccipital; they are each nearly as big as the basioccipital condyle. The condition of the opisthotic is as described for *Scomberomorus*. The vomer has an oblique articular facet on each side for attachment to the maxillary. The prefrontal is a much swollen bone broadly meeting its opposite fellow at the median line behind the ethmoid; it is pierced by the olfactory nerve. The palatine is attached to the prefrontal and the ethmoid as described under *Scomber*. The ethmoid is a thick wide bone forked or concave in front to receive the blunt premaxillary processes; its most anterior part is not at all posterior to the front of the vomer. The alisphenoids reach forward nearly to the prefrontals, and meet each other at their anterior ends above the opening to the brain case, but do not at all obstruct the opening.

The suborbital ring is incomplete. The nasals are thick, wide bones attached for their full length to the frontal and ethmoid and do not at all project beyond the latter. The symplectic from the outer surface of the skull appears to be long and cylindrical, but behind the metapterygoid and quadrate it spreads out to a broad triangular shape, and is attached to the inner surface of the metapterygoid by a deeply dentate suture. It extends downward in a channel in the quadrate. The auxiliary maxillary is well developed, and the premaxillary teeth are large and set in alveoli. The third and fourth superior pharyngeals have such a deep constriction between them on each side that they can no longer be said to form a single plate as in *Scomber*. The other head bones do not differ materially from those of the Thunninæ.

The shoulder girdle is essentially as in *Scomber*, but the pelvic girdle differs in having the subvertical plate below the horizontal plate turned outward, and a similar plate developed above the horizontal plate.

There are 45 vertebræ; it is impossible to distinguish abdominal from caudal vertebræ in the specimen at hand, as the ribs and anal

fin are detached and the two portions of the vertebral column are differentiated only by the attachment of these elements. The parapophyses are nowhere large, and the first one large enough to consider occurs on the ninth or tenth vertebra, though there are three or four bony tubercles developed anterior to this. The parapophyses bend down and join to form hæmal arches at about the middle of the abdominal cavity. The first two vertebræ bear epipleurals only. The anterior ribs fit in sockets in the vertebræ, but posteriorly where the parapophyses are developed the ribs are attached to their tips, so when the former unite to form hæmal arches the bases of the opposite ribs are brought in contact. The ribs are all directed backwards so that they lie close together. The epipleurals are developed back to the caudal keel, and are all on the centra of the vertebræ, so are remote from the ribs where the ribs drop down to the tips of the parapophyses or hæmal spines. The zygapophyses are well developed along the greater part of the column, but toward the tail they become very small and neural and hæmal processes take their place. These posterior spines are broad and flat, and each are laid firmly down over the next succeeding vertebra apparently restricting the vertical motion of the tail. A wide keel is developed on the side of the caudal peduncle, and a urostyle is present on the hypural plate. The forked caudal rays nearly cover the hypural plate from sight. The baseosts are much expanded laterally at the base of the dorsal spines. The interspinous rays of the vertical fins are crowded together and have well developed lateral wings, but they are not connected to each other by bony wings.

#### THUNNUS.

A specimen of *Thunnus alalunga* (Gmelin), 35 inches in length, from San Diego, California, and a head of a large specimen of *Thunnus thynnus* (Linnaeus), 9½ inches long, from the tip of the vomer to the end of the basioccipital, from San Francisco.

In this form the ethmoid is produced to a median angle in front, and the nasals project far anterior to it. The supraoccipital crest projects back in a spatulate process unattached below to the exoccipitals. The epiotics meet broadly, but a slender spur of bone sent down from the supraoccipital to the exoccipitals covers the suture

between them. There is a deep pit or infolding of the bone on the lower surface of the cranium between the pterotic, the prootic, and the sphenotic, just inward from the condyle of the hyomandibular. It extends upward, so that the prootic is in contact with the epiotic on the inner surface of the cranium, and the pterotic is thus completely excluded from the brain chamber. There is a large, triangular, smooth opening into the brain chamber just behind the frontal on each side of the supraoccipital crest. The alisphenoids are joined together in such a way at the median line that the anterior opening into the brain chamber is divided into a small one in front of their union and a little larger one behind. The myodome opens through a long slit in the side of a very large conical chamber in the parasphenoid. This chamber in a cranium measuring  $5\frac{1}{2}$  inches in length is an inch long and nearly half an inch broad across its mouth. The opening in its side from the myodome does not extend quite to its tip. The vomer bears an elongate patch of teeth. The suborbital bones are similar to those of *Scomberomorus*, but the cheek scales that border the eye are much thicker.

The vertebræ number 40 with the hypural plate; in this specimen as in our specimen of *Sarda* the ribs and anal fins are detached and it is impossible to distinguish the caudal from the abdominal vertebræ without them. On the fifth vertebra the first parapophysis appears; on the next three it has increased very rapidly in size, and extends straight out laterally; on the ninth vertebra its ends bend abruptly downward, and on the tenth it has united with its opposite fellow to form a broad round hæmal arch without a spine. On the eleventh vertebra a hæmal spine appears and the bases of the opposite ribs are brought in contact with each other as in the other members of its family. The inferior postero and antero-zygopophyses are equally developed, arching towards each other each as a sharp spur. There is no inferior foramina in the base of the hæmal arches. The superior zygopophyses, the ribs and epipleurals, and the other elements not mentioned are as in *Sarda*.

The skull of *Thunnus thynnus* differs from that of *T. alalunga* in having the parasphenoid developed upward in a broad wing to meet the descending wing from the basiphenoid, and a descending wing developed from the union of the alisphenoids.

## EUTHYNNUS.

A specimen of *Euthynnus pelamis* (Linnaeus), 19 inches in length, from Japan.

In this form the cranial crests, the condition of the ethmoid and nasals, the chamber in the parasphenoid into which the myodome opens are all nearly identical with these characters in *Thunnus* and *Auxis*. It differs in having the alisphenoids separated, and the vomer toothless. The large pit behind the prootic has nearly broken through the top of the cranium where the bone is very thin and

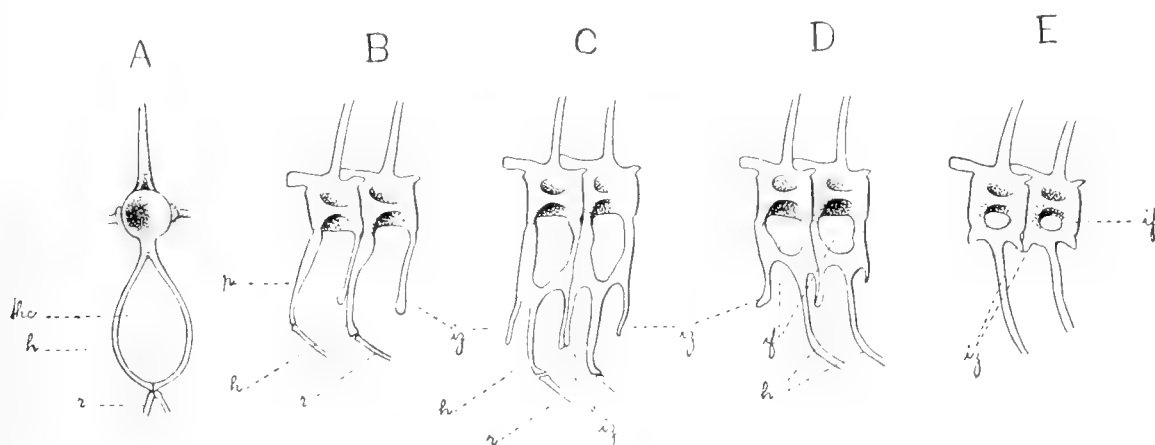


FIG. 1.—VERTEBRÆ OF EUTHYNNUS.

- A. Front view of 15th abdominal vertebra.  
 B. Lateral view of 11th and 12th abdominal vertebrae.  
 C. Lateral view of 15th and 16th abdominal vertebrae.  
 D. Lateral view of 3d and 4th caudal vertebrae.  
 E. Lateral view of 8th and 9th caudal vertebrae.  
 iz., inferior zygapophysis.  
 if., inferior foramen.  
 h. c., hæmal canal.  
 p., parapophysis.  
 h., hæmal arch.  
 r., rib.

pierced by several small holes. On top of the cranium just behind the frontal at each side of the supraoccipital crest, where in *Thunnus* is a large smooth opening to the brain cavity, the bone is irregularly broken through; the opening being much wider on one side of the cranium than on the other in the specimen at hand. There is no infolding of the bone between the prootic and alisphenoid, and there



is no opening into the myodome along the posterior edge of the lateral process of the parasphenoid as in *Auxis*.

The greatest difference between *Euthynnus* and *Thunnus* lies in the condition of the inferior vertebral processes. There are 20 abdominal vertebræ, and a like number of caudal, or a total of 41 with the hypural, the abdominal and caudal regions being more evenly divided than in *Auxis*. The lower processes of the vertebræ differ from those of *Auxis* in that the inferior foramen that is typically through the base of the hæmal arch is here enormously developed, while the hæmal arches themselves have developed to even a greater degree. The postero and antero-zygopophyses share equally in forming long slender processes between the hæmal arches. The arches spring almost directly from the body of the vertebræ, and the largest are as wide as the length of a vertebra and over twice that long. The longest diameter of the largest of the inferior foramina is considerably greater than the length of a vertebra. The first parapophysis appears on the eighth vertebra, and is scarcely developed, but the succeeding ones quickly attain a great length. Only four pairs are developed before they unite in hæmal arches. The first six or seven hæmal arches are broadly rounded at the lower median line, but posteriorly a hæmal spine is developed.

The ribs and epipleurals and their attachment to the vertebræ are as in *Auxis*. The caudal keel is as wide, but is not developed so far forward. The other vertebral processes, and all of the other elements not here mentioned are essentially as in *Auxis*.

#### AUXIS.

A specimen of *Auxis thazard* (Lacepede), from Japan, 9 inches in length.

The supraoccipital crest is as in *Thunnus* or *Sarda*, but the temporal crests run less obliquely, and merge into the general level of the supraorbital rim without reaching the orbital edge. The pterotic crest runs more on top of the cranium and forms less of the lateral cranial outline. There is a well marked depression running along each side of the supraoccipital crest from the front of the frontal to the epiotic as in *Sarda* and *Thunnus*. The myodome opens posteriorly in a very large round opening where the basioccipital and parasphenoid

are expanded to accommodate it, though there is no well separated parasphenoid chamber as in *Euthynnus* and *Thunnus*. Between the prootic and alisphenoid there is a narrow infolding of the bone to form a deep groove.

The supraoccipital widely separates the parietals, but does not altogether separate the epiotics, which meet broadly behind it. There is no opening into the brain chamber behind each frontal, and the pit behind the prootic shows no tendency to break through the top of the cranium. The lateral process of the parasphenoid attaches to the prootic above but along its posterior edge is an opening into the myodome.

The auxiliary maxillary is as broad as the maxillary and nearly half as long. The teeth on the jaws are very small; there are none on the vomer or palatines. The third and fourth suprapharyngeals are covered with moderate sized teeth, and they are a little less closely attached to each other than in *Scomber*. On the second pharyngeal the teeth are very small and set in a small patch, which is scarcely differentiated from the other dentiferous plates that cover the inner surface of the arches. The other characters of the skull, and shoulder and pelvic girdles are essentially as in *Euthynnus*.

There are 22 abdominal vertebræ and 15 caudal, or a total of 38 with the hypural. The abdominal region is very much longer than in *Scomber*, and a most extraordinary modification has taken place in some of the inferior processes of the vertebræ. This condition will best be appreciated if studied from behind forward. On the fourth caudal vertebra the processes are normal; a hæmal arch of moderate size springs directly from the lower surface of the vertebra, and terminates in a long hæmal spine. From the front of the arch the antero-zygopophysis meets the postero-zygopophysis of the preceding vertebra; each forming half of a sharp spur. On the next vertebra the antero-zygopophysis and the base of the arch have enlarged into a single, solid, bony pedicle carrying the hæmal arch away from the body of the vertebra. The pedicle gradually increases in length until at about the beginning of the posterior third of the abdominal cavity the hæmal arch is distant from the body of the vertebra a distance equal to twice the length of a vertebra. The hæmal arch is nowhere

enlarged but is in the form of a small ovate foramen. The spur of the zygopophysis is carried out with the hæmal arch and projects in front of it as it does when in the normal position on the surface of the vertebra. The spur disappears on about the third from the last abdominal vertebra, and in front of this the hæmal arch is open above so that the tip of the process is forked; each fork being now

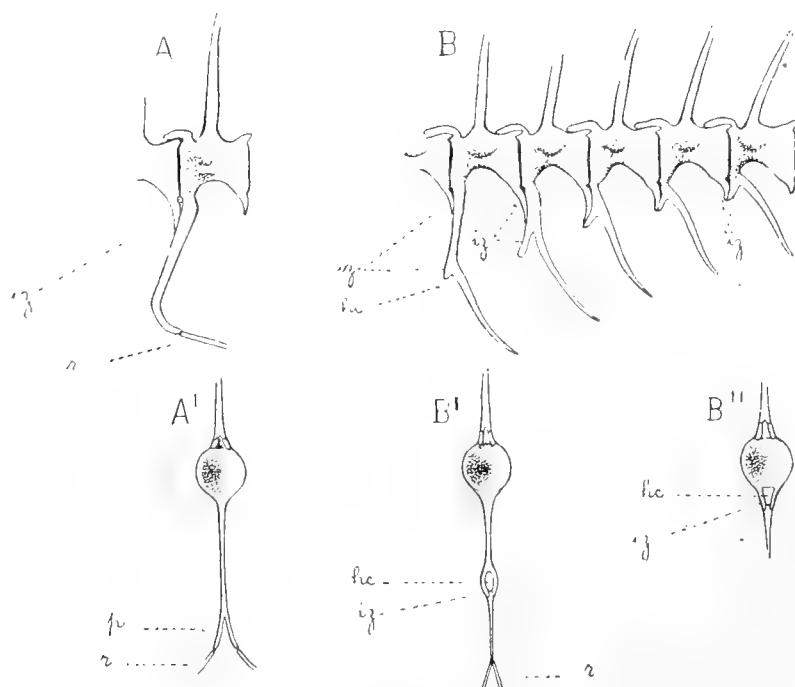


FIG. 2.—VERTEBRÆ OF AUXIS.

A. Lateral view of 17th abdominal vertebra.

A' Front view of the same.

B. Lateral view from the 1st to the 5th caudal vertebrae.

B'. Front view of the 1st caudal vertebra.

B''. Front view of the fifth caudal vertebra.

iz., inferior zygopophysis.

p., parapophysis.

r., rib.

hc., hæmal canal.

the homolog of a parapophysis. The process or pedicle holds its length to about the middle of the abdominal cavity, anterior to which it gradually grows shorter and disappears on the eighth abdominal vertebra. There are no parapophyses present that are not anchylosed with their fellows of the opposite side at the base. When the antero-

zygopophysis begins to enlarge the postero slightly enlarges to meet it, but it at once begins to grow smaller again and does not help to form the pedicle. The superior postero-zygopophyses are moderately developed, but the antero-zygopophyses are very large and reach far over them.

The ribs, epipleurals, and other elements of the trunk are as described for *Euthynnus*.

#### EXPLANATION OF PLATES.

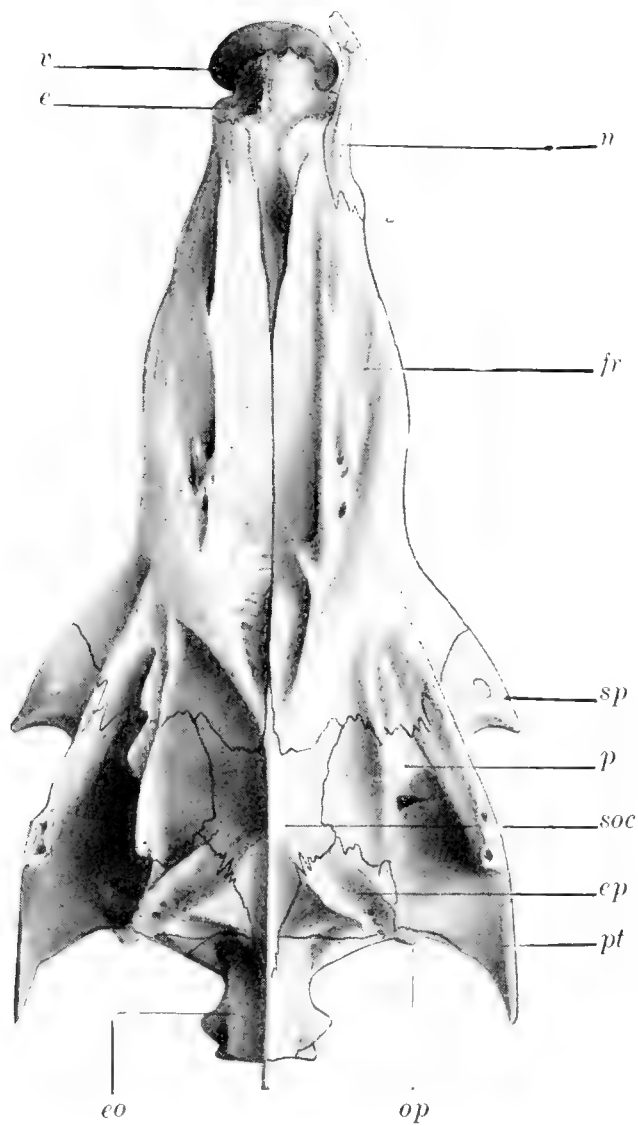
*e*, ethmoid.  
*eo*, exoccipital.  
*ep*, epiotic.  
*fr*, frontal.  
*n*, nasal.  
*op*, opisthotic.  
*p*, parietal.  
*pf*, prefrontal.  
*pt*, pterygoid.  
*soc*, superoccipital.  
*sp*, sphenotic.  
*v*, vomer.

PLATE I  
SUPERIOR VIEW OF THE CRANIUM OF SCOMBER JAPONICUS

PLATE II  
SUPERIOR VIEW OF THE CRANIUM OF SCOMBEROMORUS SIERRA

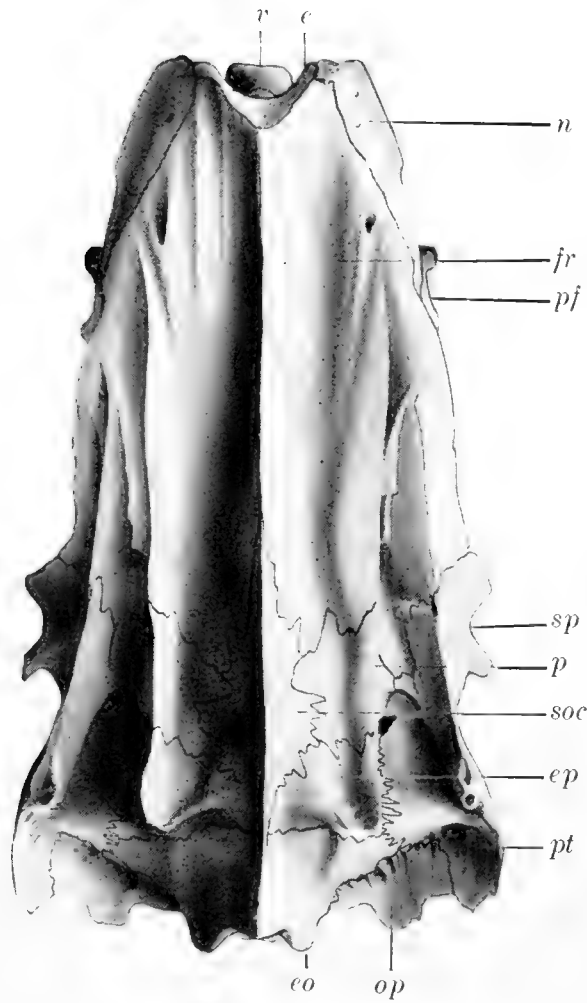
PLATE III  
SUPERIOR VIEW OF THE CRANIUM OF SARDA CHILIENSIS



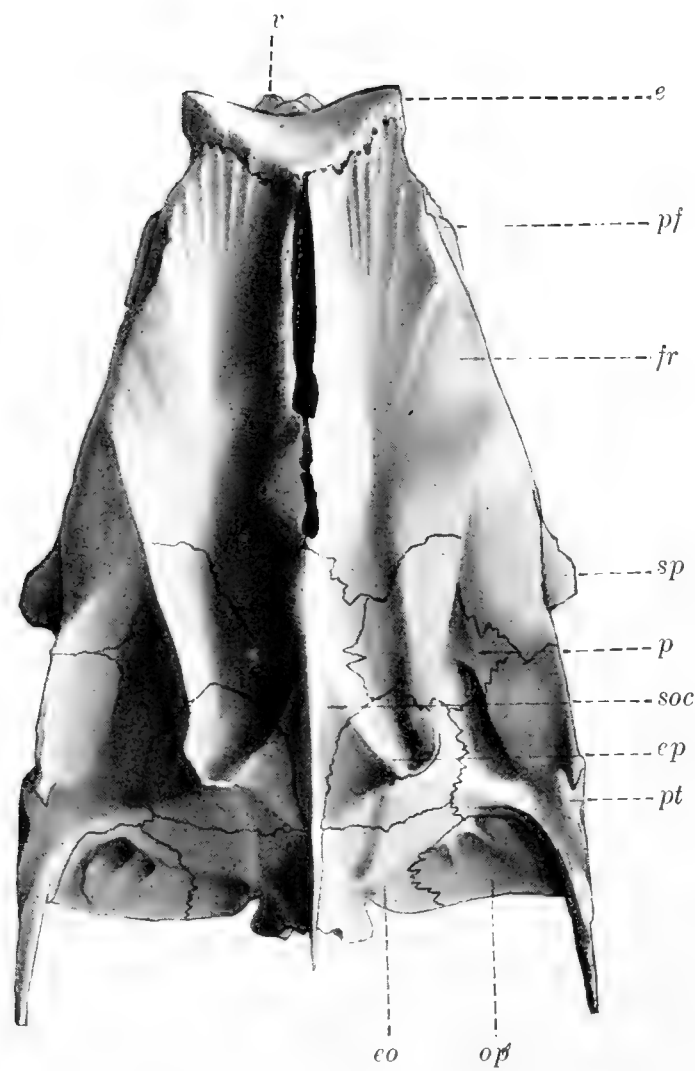














# STUDIES ON THE EARLY DEVELOPMENT OF THE HEN'S EGG.

## I. HISTORY OF THE EARLY CLEAVAGE AND OF THE ACCESSORY CLEAVAGE.<sup>1</sup>

J. THOMAS PATTERSON.

WITH 32 FIGURES.

### I. INTRODUCTION.

The period extending from ovulation to the laying of the egg is a most obvious gap in our knowledge of the development of the hen's egg. It has been the writer's desire to fill in this break, and he is indebted to the trustees of the "Elizabeth Thompson Science Fund" for a grant which made it possible to undertake the work. If the problem contained no possibilities other than that of merely filling in a gap, it is doubtful whether the work would have been undertaken, since the results could not have been commensurate with the labor involved. But it was felt that certain points, brought out in a study of the pigeon's egg by several students at the University of Chicago (Harper, '04; Blount, '09; Patterson, '09), deserved further investigation. Among these were fertilization, accessory cleavage, and gastrulation.

On account of the importance centering in gastrulation and the accessory cleavage, their discovery in the hen's egg would be of the greatest interest; for a true gastrulation has never been found in this egg, and the accessory cleavage has been neither figured nor described. We have not even known whether fertilization in the hen's egg is monospermic or polyspermic.

<sup>1</sup>Contribution from the Zoölogical Laboratory of the University of Texas, No. 103.

It is not necessary to enter into an extensive discussion of the literature on the subject of the early development of the hen's egg, for the several papers touching on this subject are well known. The studies of Duval, '84, have, perhaps, received more attention than those of any other investigator, and yet it has been demonstrated that his fundamental conclusions are incorrect, and that he was probably misled in his interpretations through the use of pathological material (Kionka, '94; Barfurth, '95; Schauinsland, '99; Patterson, '09). Kionka, '94, although figuring stages throughout the greater part of the period to be considered in these studies, does not give us a good idea of the character of the very early cleavages. Neither of these workers, nor any one of the others who have investigated these stages, has had anything like a complete series from which to draw his conclusions; consequently it is not surprising to find that the majority of the interpretations do not accord with the principles of vertebrate development, and that the more fundamental points are obscure.

The recent discovery by Guyer, '09, of an "accessory chromosome" in the male germ cells of the chicken lends unusual interest to the study of fertilization in the hen's egg, for it ought to be possible to demonstrate from the study of the mitoses of the supernumerary sperm nuclei whether or not such nuclei are dimorphic.

It was the writer's intention at first to publish the entire history, from ovulation to laying, in a single paper, but the slow rate at which material naturally accumulates makes it desirable to publish the part already completed; the remaining parts, one on maturation and fertilization, and the other on late cleavage and gastrulation, will appear later.

## II. METHODS.

Since the methods employed are essentially the same as those used in handling the pigeon egg, they need be mentioned but briefly here. The picro-sulphuric-acetic mixtures, which were found to be so excellent for fixing the pigeon egg, do not work well, for they render the yolk too hard. The picro-acetic fluid, however, although not entirely satisfactory, gives fairly good results. For preparing

surface views, a weak solution of Flemming's chromo-osmic acid is excellent and has but one disadvantage, viz., that after its use the egg usually can not be sectioned.

### III. SOME NOTES ON THE LAYING HABITS OF THE HEN.

The behavior of the hen during the breeding season would make an interesting topic for the student of animal behavior; for while one sees many evidences suggesting that domestication has wonderfully influenced the behavior of the hen, yet there are continually cropping out certain habits that evidently have been derived from her wild ancestors, and which even centuries of domestication have not completely eradicated. One of the most noticeable of these is seen in connection with the nest building. The hen never carries building material to the nest, but she often stands in the vicinity of the proposed site and makes a futile effort to get straws and feathers into the nest by tossing them over her back. In several of the other Gallinæ this same habit is observed. Many species of this group of birds are accustomed to building their nests in tufts of grass, where an abundance of material is ready at hand, and its building is a comparatively simple matter, consisting in the arrangement of the grass. Occasionally, however, other birds (*e. g.*, the quail) will engage in exactly the same futile effort as that cited above for the hen, only in a more pronounced manner.

The writer's study of the habits of the hen was not carried on with any intention of writing a paper on its behavior, but rather in order to find out if there is any regularity in its laying habits. If one is to collect eggs for the purpose of obtaining a close series of stages, it is of the greatest importance to be able to tell just when to kill the hen in order to secure a desired stage. It is only in this way that one can hope to obtain sufficient data for a correct interpretation of the history of development.

It is commonly supposed that the hen lays very irregularly, and while the writer finds this to be true for some few hens, yet in most cases he was soon able to predict the time of laying to within a few minutes. This is especially true of that class of hens laying daily. Such hens are found to lay slightly later each day, and



the difference between any two succeeding days is sometimes exactly one hour (hen 1).

## HEN 1.

Laid	April 1,	8:30	A.M.
"	" 2,	9:30	"
"	" 3,	10:30	"
"	" 4,	11:30	"
"	" 5,	12:30	"

When a hen is not laying daily the matter of determining the time is not so simple, and yet even here one can approximately predict the exact time of the laying, as can be demonstrated in the following case:

## HEN 2.

Laid	June 12,	2:00	P.M.
"	" 14,	10:00	A.M.
"	" 15,	2:00	P.M.
"	" 17,	10:00	A.M.

It is evident from these data that the hen was laying at 10:00 A.M. and 2:00 P.M. on succeeding days and then was missing a day. It was, therefore, predicted that she would lay early in the afternoon of the 18th, and since an early cleavage stage was desired, the hen was killed at 4:00 P.M. on the 17th. The stage secured is shown in Fig. 15.

There are some hens that apparently do not lay at any regular intervals, and in such it is quite impossible to predict the time of laying. As an example, I may cite the following case:

## HEN 3.

Laid	July 25,	11:00	A.M.
"	" 28,	11:00	"
"	" 30,	11:45	"
"	Aug. 1,	11:00	"
"	" 6,	9:30	"
"	" 7,	3:00	P.M.
"	" 9,	1:00	"
"	" 11,	12:30	"
"	" 13,	2:00	"
"	" 16,	1:30	"
"	" 18,	1:00	"

Killed August 19, 5:00 P.M. (secured an early cleavage stage).

There are but few hens that ever lay before 8:00 A.M. or after 4 P.M. It is evident, therefore, that a hen in laying daily will eventually come to the 4 o'clock period, and will then miss a day (sometimes more) before beginning a new set. Indeed, this is true for all hens whether laying regularly or irregularly, for they lay in a sort of rhythm. In the case of irregular laying, cited above (hen 3), the eggs laid from the 25th of July (when the observations were begun) to the 1st of August, are the last of a set in which the hen was laying approximately every other day; while those laid from August 6th to 18th constitute another set. Evidence that the eggs will be laid in sets can be obtained by an examination of the ovary, which shows several graduate series of ovarian eggs.

It will be evident from the above considerations and data that if one is to secure a close series, it is necessary to study each hen individually and while this involves a great amount of labor, yet it is the only way in which one is able to meet with any success.

The collecting of the above data has another advantage besides that of aiding in securing a close series, for it makes possible the determination of the rate of development of the different stages. The time occupied by the egg in passing down the oviduct has been variously estimated at from eighteen to twenty-four hours, and even as high as thirty-six hours. This seems like a wide variation, and in taking up this work, the writer was prepared to find the normal time more constant than is indicated in such estimates.

The writer finds that in a hen kept under normal conditions, the egg traverses the entire length of the oviduct in about twenty-two hours. The time occupied in the different portions of the oviduct is as follows: Glandular portion, three hours; isthmus, two to three hours; uterus and laying, sixteen to seventeen hours.

As just stated, these estimates were made on hens kept under normal conditions; that is, hens that were given the freedom of the barnyard. It is possible to lengthen the time beyond the twenty-two hours by disturbing the hen when she is about to lay, and on one occasion the writer was able to delay the laying of the egg for twenty hours. When it was finally deposited an examination revealed the fact that it was in a stage of development equal to about twenty hours of incubation. This would account for the high esti-

mates of other writers, and perhaps also for those cases reported in the literature, where a freshly laid egg is said to contain an embryo with a well-developed vascular system. The writer is convinced that any appreciable extension beyond twenty-two hours is not due to an increase in the length of time that it takes the egg to traverse the reproductive passage, but rather to a retention of the egg in the lower part of the oviduct, on account of some influence inhibitory to laying. The writer has found, however, slight variations in the length of time, but these are probably to be correlated with the variations in length of the oviduct in different hens.

When once it has been established that the time occupied by the egg in its passage through the different parts of the oviduct is practically constant, we are then in a position to determine the rate of development; because we need only to note the stage of development in eggs taken from the different parts, and from the data thus collected determine the time elapsing between any two stages. The following table will give the reader an idea of these estimates, which were determined from a study of hens laying daily (about one hour later each day). The table also gives the exact time of each of the stages described in the rest of the paper.

TABLE I.

Hen.	Last egg laid.	Succeeding taken from the oviduct.	Age.	Position in oviduct	Stage	Fig.
No. 1	1:30 A. M. May 28	4:00 P.M., May 28	2½ hrs.	11 inches from the infundibulum	pre-cleavage	5
No. 2	8:30 A.M., Aug. 10	2:30 P.M., Aug. 10	3 "	just entering the isthmus	two-celled	1
No. 3	8:30 A.M., Apr. 13	2:30 P. M., Apr. 13	3¼ "	in the isthmus	four-celled	12
No. 4	10:00 A.M. Sept. 1	5:00 P.M. Sept. 1	4 "	" " "	eight-celled	13
No. 5	11:00 A.M., Aug. 4	6:30 P.M., Aug. 4	4½ "	" " "	fifteen-celled	21
No. 6	12:30 P.M., July 31	8:15 P.M., July 31	4½ "	" " "	thirty-two celled	
No. 7	8:45 A.M., July 30	4:45 P.M., July 30	5½ "	in shell gland	64 cells in surface view.	25
No. 8	9:30 A.M., Aug. 7	7:30 P.M., Aug. 7	7 "	" " "	154 cells in surface view.	30
No. 9	8:00 A.M., Aug. 27	7:00 P. M., Aug. 27	8 "	" " "	346 cells in surface view.	32

## IV. FUNCTIONS OF THE OVIDUCT.

The whole reproductive apparatus is a most delicately adjusted mechanism. The primary function of its oviducal portion is to transmit the egg from the body cavity to the exterior, but in the course of its evolution it has taken up several other functions, such as transmitting and storing sperms and the secreting of the accessory layers around the egg. The co-ordination between the infundibulum and the ovary is often very exact and delicate, but it is in the birds that we see this co-ordination reaching its highest degree of perfection. Coste describes the infundibulum as actually embracing the ovum in its follicle at the time of ovulation, and the writer has been able to confirm his statement by several observations. Coste believed that the infundibulum exerted some pressure on the follicle, and it may be that this is the direct cause of ovulation. Indeed, it is highly probable; for while ovulation may take place without the direct assistance of the oviduct, as in the lower vertebrates, yet the weight of evidence supports the opposite view. We have been able to show (in a paper not yet published) that the follicular orientation is preserved in the oviduct, and furthermore that this preservation probably occurs only when ovulation is directly caused by the activities of the infundibulum.

This explanation of ovulation also gives us the key to the solution of another problem, viz., why it is that normally but a single egg is found in the oviduct at a time. If we examine the oviduct of a hen that is laying daily, some time before the deposition of an egg, it will be found to be inactive; but an examination shortly after laying, reveals the fact that the oviduct is in a state of high excitability, with the infundibulum usually clasping an ovum in the follicle. In one case it was embracing a follicle containing a half-developed ovum, and with such tenacity that a considerable pull was necessary to disengage it. It seems certain, therefore, that the stimulus which sets off the mechanism for ovulation is not received until the time of laying, or shortly thereafter. So long as there is an egg in the lower part of the reproductive passage the infundibulum apparently does not clasp the ovum, and a second egg is thus prevented from entering the oviduct.

## V. FERTILIZATION.

Since it is intended to describe in detail the process of fertilization, the writer wishes here to make only a brief statement concerning the time of its occurrence. Harper, '04, has shown that fertilization in the pigeon's egg takes place immediately after ovulation, when the egg is in the region of the infundibulum. The writer finds the same to be true of the hen's egg also. Eggs taken from the upper part of the oviduct at distances varying from one to twelve inches from the infundibulum, give all the stages of development from maturation to the formation of the first cleavage spindle.

We have shown above that the egg is about twenty-two hours in passing down the oviduct, and since fertilization takes place immediately after ovulation, it is obvious that it occurs approximately twenty-two hours before the time of laying. Throughout this paper we shall, therefore, determine the "age" of any particular stage from this estimated time of fertilization.

## VI. THE TWO-CELLED STAGE.—THREE HOURS.

The first cleavage furrow makes its appearance just as the egg is entering the isthmus, about three hours after the estimated time of fertilization, and by the time the inner-shell membrane can be recognized as an extremely thin sheet covering the albumin, the furrow is well developed and covers a distance equal to about one-third the diameter of the area of primary cleavage. The furrow is usually situated in the central part of the disc (Fig. 1).

Any mention of the first cleavage furrow calls to mind the question of the relation of the plane of this furrow to the longitudinal axis of the later embryo. Of the five cases so far obtained, in which it was possible to determine absolutely the plane of the first furrow, only one showed it coinciding with the long axis of the future embryo. This would seem to indicate clearly that the plane of the first furrow does not necessarily parallel the median axis of the embryo. It may be, however, that in the case of each of the eggs mentioned, we were dealing with one in which the axis of the later embryo would be abnormal; that is, it would not meet the chalazal axis at right angles. Duval, '84, has pointed out that a

certain number of hen's eggs show abnormal relations existing between the two axes, and the writer has found a similar condition in the pigeon egg. In each of these species the percentage of abnormal axes was found to be small. It seems highly improbable, therefore, that four out of five eggs taken at random, and in the two-celled stage, would later show abnormal axes. The final answer to the question, however, could only be obtained by studying a two-celled stage and noting the plane of the furrow, and then after incubating the egg until the axis of the embryo became visible, it would be possible to determine the point in question. This would necessitate a much more extensive study than the object of this paper calls for. The problem, furthermore, has lost much of its earlier significance, inasmuch as it has not proved to be a fundamental law of development.

In sections taken transverse to the first cleavage furrow (Fig. 2) the membrane is seen to cut almost through the fine granular portion of the disc, and is peculiar in that it arises from a *membrane plate*, which, at both sides, is continuous with the perivitelline space. In section the membrane does not extend down from the membrane plate as a straight line, but is wavy (Fig. 3); and while this condition may be the result of unequal contraction of the materials, caused by the fixing and hardening fluids, yet it obtains for all of the earlier membranes.

Another point of interest brought out by the section (Fig. 2) is the depression in the surface of the disc just above the cleavage membrane. This is, of course, the cleavage furrow, which in this egg stood out with remarkable clearness in the living condition, but in most eggs it is practically wanting (Fig. 11). The lack of a furrow is the cause of the indistinctness of the early cells. In this respect, the early cleavages of the hen's egg differ greatly from those of the pigeon's egg, for in the latter their clearness is such as to permit photographing the living cells, while in the former, photographs are impossible, except in a few cases.

After the division of the first cleavage nucleus the daughter nuclei migrate peripherally in a line lying at right angles to the cleavage membrane, and are always elongated in the direction of motion. In

this egg they showed signs of preparation for the next division when they had reached a distance from the membrane equal to 0.175 mm.

*Polyspermy.* A close study of surface views of two-celled stages has failed to reveal any trace of the "accessory cleavage," which is such a characteristic morphological feature of the early pigeon blastoderm. It would be a great mistake, however, to conclude from this that fertilization in the hen's egg was monospermic, for a study of the sections brings to light the fact that ordinarily five or six supernumerary sperm nuclei are in the egg, and, as we shall see later, some of these may migrate to the periphery of the area of primary cleavage and there give rise to a *rudimentary accessory cleavage*.

In one egg (Fig. 5), which is in a preeleavage stage of development, twenty-four extra sperm nuclei are found. This high number is very unusual, and led the writer at first to assume that the egg was abnormal. All the evidence, however, is against this assumption. In the first place, it can not be said that the physiological condition of the egg was such as to favor the multiplication of the sperm nuclei soon after their entrance into the egg; for if this were the case there ought to be evidences of nuclear multiplication, but not a single sperm nucleus gave any sign of undergoing division. In the second place, the egg in all probability was normal in so far as undergoing normal development is concerned, for the cleavage nucleus was in the act of producing a spindle at the time when the egg was fixed, and other eggs from this hen underwent normal development when incubated.

In the light of these facts it seems evident that in some few eggs a comparatively large number of sperms may enter. In such eggs this may be due to a greater attraction between the protoplasm of the disc and that of the sperms than ordinarily exists; or to a failure of the inhibitory agencies to become operative quickly enough after ovulation to prevent their entrance.

In the egg from which Fig. 1 was made only five supernumerary sperm nuclei are present, and none of these had reached the margin of the disc at the time when the egg was fixed, but all are located centrally. Two of the nuclei are situated quite superficially in the disc, while three have passed down deep and are resting on the coarse granular yolk (Fig. 9). One of the nuclei has undergone division

twice, and produced a "nest" of four nuclei (Fig. 6, *sn*). It is difficult to determine whether or not such nuclei later migrate from the nests to the margin of the primary area and there participate in the production of the accessory cleavage. The writer believes not, because nests containing many small fragments of nuclei are found in slightly later stages, and such nests are located in the same position as the earlier ones. This would seem to indicate that the nuclei, after sinking down into the coarse granules, continue to divide and fragment, finally disappearing altogether. If this be true, then we see the beginning of the degenerative agency which will cause all of the supernumerary nuclei to disappear.

An egg showing a case of fragmenting nuclei is outlined in Fig. 10. It is a three-celled stage, and the degenerating nucleus is close to the margin of the primary area. All of the sperm nuclei, excepting one, are located much more peripherally than in the preceding egg (cf., Figs. 9 and 10). The difference in position of the two sets indicates the distance traversed by the nuclei during the time intervening between the two stages.

A section of the egg figured above gives one a good idea of the character of the blastodisc in the early stage (Fig. 11). It also demonstrates the point made above, that very often the first cleavage membranes are not accompanied by a cleavage furrow.

This egg furnishes still other points of interest, for in fixing it, not all of the albumin was removed, and the thin chalazipherous layer adheres to the vitelline membrane. Embedded in this layer and next to the membrane are about ninety sperm heads, none of which is located peripherally to the terminal ends of the cleavage membranes (Fig. 8).

The presence of the sperm heads in such large numbers, together with their position at the central part of the disc, is important. It can not be said that the scarcity of sperm nuclei in the disc (as compared with the number found in the pigeon egg) is to be accounted for by the lack of sperms in the oviduct. Their location in the central part of the disc only, shows that they must be attracted there by a force which is strongest at the central point, and which gradually diminishes toward the periphery. The attraction between the protoplasm of the disc and that of the sperms is evidently neu-



tralized suddenly, because sperms are found lodged in the vitelline membrane, as though they had been stopped in the very act of entering the egg (Fig. 7).

#### VII. THE FOUR-CELLED STAGE.—THREE AND ONE-FOURTH HOURS.

The four-celled stage is produced by a vertical division in each of the blastomeres of the two-celled stage, and the two furrows meet the first one approximately at right angles (Fig. 12). While the division in one blastomere may slightly precede that of the other, usually they occur simultaneously. It has been stated that the point where the second furrows meet the first is situated eccentrically, the displacement being toward the posterior border of the blastoderm. It is not uncommon to find eggs with the center of the cleavage eccentric, but the displacement may be in any direction from the center. The writer does not believe, therefore, that any importance can be attached to the eccentricity of cleavage.

The rudimentary accessory cleavage makes its appearance in the four-celled stage, and in the egg shown in Fig. 12 there are three such furrows present. These cut across the margin of the area of primary cleavage, and their planes are approximately radial. In most eggs the furrows lie entirely without the margin. This blastodisc shows, in addition to the three accessory furrows, two other small ones lying well within the margin, but their position in the anterior blastomeres makes it clear that they are the approaching divisions of these two cells.

#### VIII. THE EIGHT-CELLED STAGE.—FOUR HOURS.

In the formation of the eight-celled stage, the third division furrows, at least in some cases (Fig. 16), tend to remain regular; that is, each of the third furrows is vertical and meets the second furrow at right angles. There is thus produced two parallel rows of four cells each. In the majority of eggs, however, the form of the cleavage in this stage apparently is not regular, though probably if it were possible to follow the divisions in the living cells it would be found that there was considerable regularity. The cells do not always divide simultaneously, and since there is a tendency for the

early blastomeres to flow together, it may be that the original relationship between the cleavage planes is modified. If this is not the case, then the variation in the form of cleavage which characterizes the later stages is anticipated in the eight-celled stage.

As in the two- and four-, the cells of the eight-celled stage are not true cells, in the sense that they are not completely delimited by cell walls, but are open to the periblast both below and peripherally. Occasionally, however, one of the blastomeres may be surrounded (in surface view) by cell walls (Fig. 13). We have, therefore, two regions of cleavage, in which the cells are usually designated as central and marginal.

The connections between both the central and marginal cells with the periblast are very clearly brought out in the section (Fig. 18), which also gives one a clear idea of the beginning of the horizontal cleavage planes. These planes not only separate the blastomeres from the underlying or central periblast, but also mark the position of the future segmentation cavity. Such an interpretation for the origin of the cleavage cavity is not in accord with the account of Duval and others. Duval contends that a very narrow space situated between a single superficial layer of cells and the deeper cells represents the segmentation cavity, and, furthermore, that the deeper cells are derivatives of the deeper parts of the disc, and have arisen by additions upward to the parts already segmented. Duval's view has been shown to be untenable for the pigeon's egg, and I find it is also incorrect for the hen's egg; but exactly the same thing occurs in the latter egg as described for the pigeon's egg by Miss Blount. The increase in the number of cell layers in the disc is not brought about by the addition upward of cells from the underlying material, but by the appearance of horizontal cleavages, which occur between the segmentation cavity and the surface of the blastodisc and thus the large central cells are cut up into a number of cell layers.

The accessory cleavage remains distinct up to the eight-celled stage (Figs. 14, 16, 13), and in the reconstruction of a series from the seven-celled stage there are shown ten supernumerary sperm nuclei (Fig. 14). Seven of the nuclei can be arranged into three groups, indicating that originally but five sperms entered the egg and not ten. Four of the nuclei are in the last stages of degenera-

tion, while two are associated with a rudimentary accessory cleavage furrow. The furrow was clearly visible in the living egg, where it appeared as a shallow groove. In section it is characterized by having a broad shallow depression, at the bottom of which is found a distinct membrane plate of exactly the same appearance as that of an early primary cleavage furrow, and differs from the latter only in the absence of a membrane (Fig. 19, a. f.). It is, therefore, rudimentary, and the presence of two supernumerary sperm nuclei in its immediate vicinity leaves no doubt as to the interpretation that it is an accessory furrow. One of the nuclei (Fig. 19, s. p., nucleus on right) has passed down into the coarser granular yolk and undergone almost complete fragmentation, and this may be the reason the furrow never proceeds to the point of forming a membrane.

In addition to the rudimentary cleavage this egg also presents an interesting case of *horizontal accessory cleavage* (Fig. 4, c.). In reality this is not a cell division, because there is associated with the cleft but a single nucleus, which lies just below the cleft. Such cases would seem to be attempts at cell formation without the accompanying nuclear division. They are not of any great importance, as but two examples have been found.

So far the writer has not observed the accessory cleavage after the ten-celled stage, and in all probability it completely disappears shortly after this period. It seems highly probable that many of the sperm nuclei degenerate soon after their entrance into the egg. The degeneration occurs when they pass down into the coarse granular yolk; but so long as they remain superficially situated they seem to possess the power of migration. Undoubtedly, there are not a few eggs in which all of these nuclei degenerate before reaching the margin, and hence such eggs would at no time show an accessory cleavage. The writer does not believe, however, that this would account for the failure of previous investigators to discover the accessory cleavage of the hen's egg. Only a few of them have described the four-celled stage, and, so far as we are aware, none have figured the eight. The brief period during which they would most likely observe this peculiar form of cleavage is, therefore, the one to which they have given least attention. Furthermore, the acces-

sory cleavage, except in a few cases, is extremely difficult to detect in the living egg; and it is only after the use of Flemming's fluid that it becomes clearly demonstrable. Its difficulty of demonstration is further increased by the fact that the furrows most often occur in radial planes, thus leading the observer to believe that they are only the terminal ends of the primary cleavage furrows. We have been unable to find a satisfactory explanation as to why the clefts take this radial direction, unless it be that they follow the course of least resistance. Not all of the accessory cleavages, however, have their furrows lying in radial planes, for the writer has found several cases in which they were lying in various planes; and, furthermore, he has observed two very clear cases of completely formed accessory cleavage cells (Figs. 13 and 17).

The contention of Blount that the accessory cleavages in the pigeon's egg completely disappear and, therefore, these cells take no part in the formation of the embryo, receives a full confirmation from these studies. The demonstration of that conclusion is even clearer in the hen's egg than in the pigeon's egg. In the hen's egg the marginal cells never become closed, thus indicating that their closing in the pigeon's egg must be in response to a stimulus received from the numerous accessory cleavage cells. Probably the closed margin cuts off influences which emanate from the accessory cells, and which might interfere with the normal development of the blastoderm.

At best the accessory cleavage in the hen's egg is but a weak attempt at cell formation, which has become inhibited, shortly after its initiation, by the degenerative tendency of the accompanying nuclei.

#### IX. FIFTEEN TO SEVENTEEN CELLS.—FOUR AND ONE-HALF HOURS.

In the stage consisting of approximately sixteen cells there are four or five central and eleven or twelve marginal ones (Figs. 17 and 20). The central cells increase by the cutting off of the inner ends of the marginal cells, while the latter multiply by the formation of radial furrows. In some cases one can still see a tendency in the form of the cleavage to remain regular. In Fig. 20 the anterior

half shows exact regularity, there being just eight cells, but the posterior half is slightly irregular, with nine cells.

There are no accessory cleavages in this stage, but a large number of short radial furrows lie just inside the margin of the primary area (Fig. 20). These furrows can be seen in the living egg, but are brought out more clearly in osmic acid preparations. Under the higher power of the microscope they are seen to be due not so much to the formation of furrows as to the arrangement of the granules. In the median lines they are composed of fine granules, while to either side are several rows of larger granules. Occasionally one can detect a membrane, which appears as a delicate thread running through the median streak of fine granules.

At first sight it might seem that these furrows represent an abundant accessory cleavage, but such is not the case. In the first place, the furrows are situated entirely within the primary area, while the accessory furrows lie without the area. In the second place, there are no nuclei directly associated with these short furrows. They are simply the beginnings of the peripheral extensions of the primary cleavage furrows; for it will be noted that for the most part they lie either in the same radial planes with the primary furrows (marginal), or in positions where the next divisions of the marginal cells will soon occur. This interpretation is fully substantiated when slightly later stages were studied, when it was found that not only are the short furrows greatly diminished in numbers, but that the primary furrows now reach the margin of the primary area (Fig. 15), and at the same time the marginal cells are practically double in numbers.

These short furrows do not occur in all blastoderms, but when they do appear it is at the sixteen-celled stage, though they may last until comparatively late cleavage stages (Fig. 30). In significance these furrows indicate that the cytoplasmic division of the marginal cells is felt at the margin of the primary area earlier than in regions lying somewhat more centrally; and the attempted division at the margin probably immediately follows that of the marginal cell nucleus, while the intervening space awaits the approach of the central portion of the primary cleavage membranes.

In median sections of the fifteen-celled stage the horizontal cleavages have progressed to the point of effecting a complete separation of the central cells from the central periblast (Fig. 22), and these cells, are therefore, completely delimited by cell walls. The segmentation cavity is expanding laterally beneath the marginal cells by the extension of the horizontal clefts (Fig. 22, on left), and will eventually increase in depth by the accumulation of fluid within it.

#### X. THIRTY-TWO TO THIRTY-FIVE CELLS.—FOUR AND THREE-FOURTHS HOURS.

In this stage (Fig. 23) the central cells have begun to multiply more rapidly than those of the margin, and the two kinds are now of equal numbers. The form of the cleavage is irregular, and, therefore, very variable in different eggs. At the posterior border of the blastoderm are seen two short radial furrows (Fig. 23, *m*), and while it is possible that there were more at an earlier period, yet probably this is an egg in which there were never many; because if numerous at an earlier period of development, the primary cleavage furrows should give some evidence of having extended to the margin of the primary area, and it will be noted that they fall quite short of reaching the margin.

The median section of a blastoderm, which showed thirty-two cells (sixteen marginal and sixteen central) in the living egg, is seen in Fig. 24, A. The cleavage cavity is especially well developed, and the one-layered condition of the blastoderm is unusually clear. The vertical cleavage separating the two cells which lie slightly to the left (anterior) of the center has not completely cut through to the cavity. Such a connection between adjacent cells is very slender, and, in this particular case, is absent in a few sections lying to either side.

While the planes of the early cleavages are, for the most part, vertical (that is, meeting the surface of the blastoderm at right angles), yet some of the planes take an oblique course. Under such conditions it is not difficult to find many places where the blastoderm appears to be two cells thick; especially is this true of sections that are taken some little distance to either side of the median line (Fig.

24, B). Here, the lower portions of the two cells (on right) appear to be "buds" or outgrowths from the floor of the cavity; but if the succeeding sections on each side are carefully examined, it will be found that such "buds" are nothing more nor less than the lower ends of cells whose upper portions reach the surface of the blastoderm in another section—and vice versâ, the cells that appear to form a free upper layer are found to have portions extending obliquely downward to, and connecting with, the floor.

This point is of considerable interest, because a failure to appreciate its import probably has been the source of error on the part of some embryologists (*e. g.*, Duval), who have stated that cells are cut off from the floor and are added upward to the blastoderm, thus contributing to its increase in thickness. The deception arising from the appearance of these buds is all the more striking in the cases where the nucleus of the cell concerned lies deep enough to be included in the "bud" (*e. g.*, Fig. 24 B, *n*). In some sections both the upper and lower portions of the cell are included (Fig. 24 B, *c*), and in such the connection of the cell with the floor is clearly shown. In later stages the connection will be severed by the extension of the horizontal cleavage planes.

The accessory cleavage has entirely disappeared by the thirty-two celled stage, but occasionally a supernumerary sperm nucleus will be found. The sections of the egg considered just above were subjected to a very careful examination for the purpose of determining how many such nuclei were present. This study gave the following results: In the sixteen central cells eighteen nuclei were found, but three of the cells had two nuclei each; that is, the cytoplasmic division of these three cells had not yet taken place. In the sixteen marginal cells, together with the surrounding periblast, nineteen nuclei were found, and two of the marginal cells had two nuclei each. The extra nucleus was found far out in the periblast—so far out, indeed, that there is no possibility of its being considered as a derivative of a marginal cell nucleus. It must be, therefore, a sperm nucleus. This could have been determined without counting the other nuclei, for the one in question is in an advanced stage of degeneration, and rapidly disappearing.

It is certain, therefore, that not only does the accessory cleavage

disappear at an early cleavage stage, but the extra sperm nuclei also disappear early. The writer has never found sperm nuclei after the thirty-two-celled stage.

#### XI. SIXTY-FOUR CELLS IN SURFACE VIEW.—FIVE AND ONE-HALF HOURS.

This stage brings out more clearly than any we have so far considered the method by which the region of central cells increases. At the inner ends of many of the marginal cells are large central ones, which have been just recently cut off; and located centrally to these are smaller cells (Fig. 25). The number of central cells increases, therefore, in two ways: First, the central region grows at the expense of the marginal cells, and in this manner the central region gradually extends peripherally; second, the central cells thus formed multiply *inter se*. If we compare this stage with the preceding one (Fig. 23), in which there were seventeen cells in each region, it will be seen at once that while the number of marginal cells has increased but six, the central ones have increased twenty-four (in surface view). This comparison becomes all the more striking when it is stated that the central region averages two cells in depth, due to the formation of horizontal clefts, so that there are probably a total of some eighty central cells, or an increase of sixty-three. The central cells multiply, therefore, more than ten times as rapidly as the marginal ones.

The manner in which the central region becomes more than a single layer deep is made clear in a study of a section of a blastoderm in this same stage of development (Fig. 26). The segmentation cavity is very distinct and above it the original one-layered disc (see Fig. 22) is now two cells deep; and this condition has been brought about not by the addition of cells from that portion of the disc lying beneath the cleavage cavity, as supposed by Duval, but entirely by the formation of horizontal clefts in the cells lying above the cavity. As we might have expected, the method of increase in cell layers is identical with that of the same process in the pigeon blastoderm. At this stage there is no possibility of cells being added to the disc from the central periblast, because that region is entirely void of nuclei.



On account of the obliquity of the so-called horizontal clefts, the central cells are characterized by great irregularity both in shape and size. Many of them are shaped like squamous epithelium, and often give the appearance of stratified epithelium in section.

A detailed drawing of the anterior end of a section will show the manner in which the marginal cells add their products to the central area (Fig. 27). The cleavage membranes cut down deep into the disc, and at their terminal points the horizontal clefts begin to spread beneath these large cells, thus separating them from the underlying portion; when this is accomplished, the large cells are split up into smaller ones by vertical and horizontal divisions (on right of Fig. 27).

## XII. ONE HUNDRED AND FIFTY-FOUR CELLS IN SURFACE VIEW.— SEVEN HOURS.

During the next hour and a half the marginal cells undergo but few divisions, but the central ones multiply very rapidly, and since they receive but few additions from the marginal cells, their increase must be due to their own activity in division. This results in producing a large number of small cells in the central area, and the smallest cells lie at the very center of the blastoderm (Fig. 30). It would seem, therefore, that the early cleavage of the hen's egg follows the rule which states that the time occupied between any two successive cleavages grows shorter and shorter as the volumes of the cells decrease.

The average depth of the central part of the blastoderm at this period is about three cells (Fig. 31). The cleavage cavity, although not so distinct as in the preceding stage, is, nevertheless, clearly recognizable and there are no connections between its floor and the lower cells of the blastoderm. The anterior and posterior ends of the section show different conditions in the character of the cells. At the posterior end there are three large cells, in addition to the marginal one, which have not been broken up by horizontal clefts. At the anterior end, on the other hand, all of the cells, except the marginal, have undergone division. This difference is probably only a local condition, and, therefore, is not fundamental.

The periblast still remains free of nuclei, but the marginal cell nuclei are beginning to show a tendency to migrate farther peripherally than usual (Figs. 28 and 29).

### XIII. THREE HUNDRED AND FORTY-SIX CELLS IN SURFACE VIEW.— EIGHT HOURS.

The final stage that we shall consider in this paper is shown in Fig. 32. During the hour intervening between this and the previous stage the marginal cells have added many more cells to the central area than at any former period of like duration, and consequently the (radial) length of the marginal cells has greatly decreased, and their furrows now are beginning to cut out into the periblast.

This stage is one of the most important of all the early cleavages, because it represents the transitional period between the "unorganized" and "organized" periblast; but we shall not consider the sections at this time.

### XIV. GENERAL SUMMARY.

The absence in this paper of comparisons between the development of the hen's egg and that of other vertebrate eggs is not due to a lack of appreciation of the importance of such comparisons, but rather to the fact that in the main these have been pointed out for the corresponding stages of the pigeon's egg. In this connection the writer wishes, therefore, to confine himself to emphasizing the close similarities between the development of the hen's egg and that of the pigeon, although to those who have followed closely the work on the latter egg this may seem unnecessary.

Exact agreement in all details of development, even in the eggs of two species as closely connected as those of the hen and the pigeon, is not to be expected, but the fundamental processes should certainly agree. And such has proved to be the case. The minor differences in development of these two forms have to do primarily with time relations; for although the eggs of these two species are in about the same stage of development at the time of laying, yet the pigeon's is forty-one hours old and the other's twenty-two. The homologous processes, therefore, necessarily do not occur at exactly the same time after fertilization.

The more important comparisons are as follows:

1. The process of fertilization (that is, the entrance of the sperm) in each egg occurs immediately after ovulation, when the egg is in the region of the infundibulum.

2. At the time of fertilization in the pigeon's egg, from twelve to twenty-five supernumerary sperm nuclei enter the egg. In the hen's egg only five or six such nuclei are found (except in one case where twenty-four were present).

3. Upon their entrance into the egg these sperm nuclei, in each egg, migrate toward the periphery of the disc. In the pigeon's egg the nuclei, on reaching the margin, become active, divide and give rise to an accessory cleavage, which disappears between ten and twelve hours after fertilization. In the hen's egg some of the supernumerary nuclei pass down into the deeper portions of the disc and there undergo complete fragmentation; others may succeed in reaching the margin, and there give rise to a rudimentary accessory cleavage, which disappears shortly after the eight-celled stage, or between four and five hours after fertilization.

4. In the pigeon's egg the marginal cells become closed and remain so throughout the period occupied by the accessory cleavage. In the hen's egg the marginal cells always remain open to the periblast both below and peripherally. This would seem to indicate that the condition of a closed marginal cell in the pigeon's egg is to be correlated with the presence of a large number of accessory cleavages. Perhaps it is for the purpose of cutting off some influence emanating from the accessory sperm nuclei.

5. In neither egg does the direction of the first cleavage plane, or the eccentricity of cleavage, if present, seem to bear any constant relation to the axis of the future embryo.

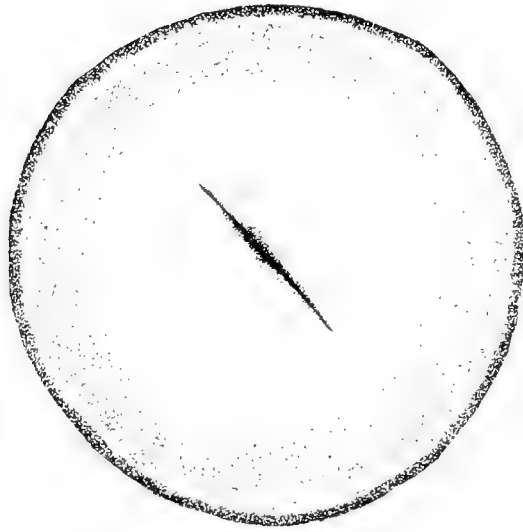
6. Immediately after the disappearance of the accessory cleavages and their accompanying nuclei in the pigeon's egg the marginal cells open to the periblast, and their nuclei divide and some of the daughter nuclei migrate into the periblast and "organize" it. In the hen's egg there is a period of from two to three hours after the disappearance of the accessory sperm nuclei during which the periblast is void of nuclei of any kind.

7. In each egg the first horizontal cleavage plane marks the position of the segmentation cavity.

AUSTIN, TEXAS, November 8, 1909.

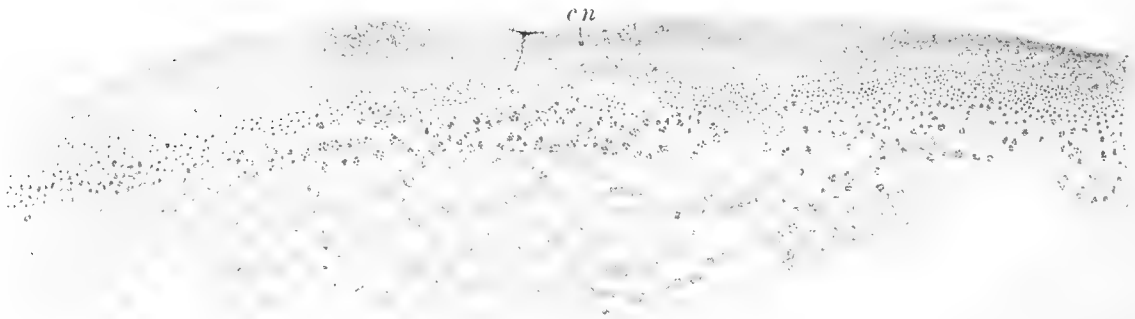
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1

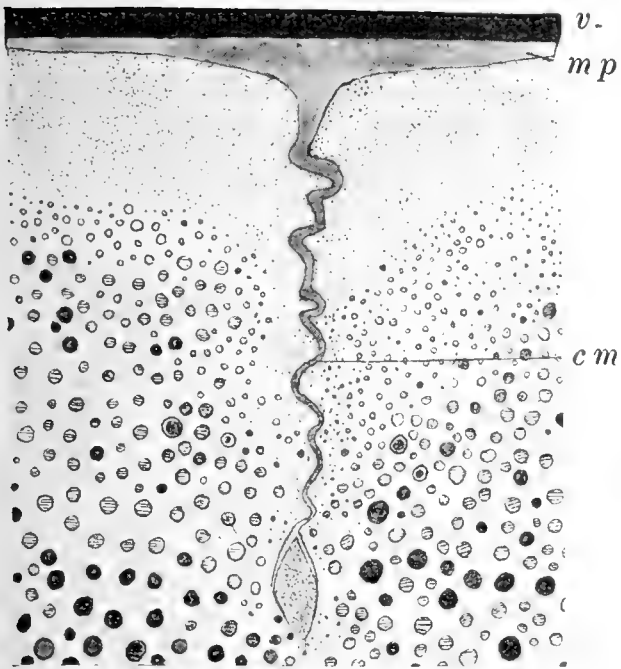
FIG. 1.—A two-celled stage, which was drawn from a free-hand sketch and measurements of the living egg.<sup>2</sup> For the history of this egg see Table 1, hen No. 2.  $\times 18$ .



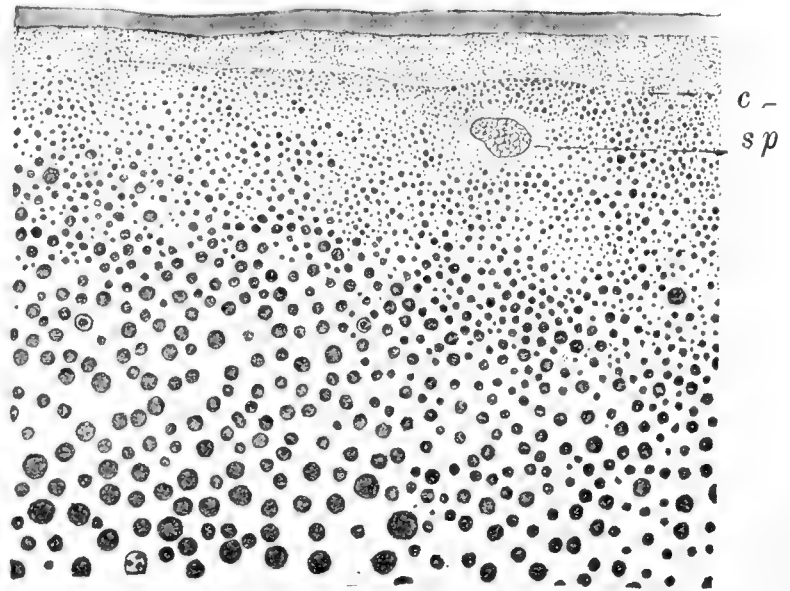
2

FIG. 2.—A median section taken transverse to the furrow of the egg illustrated in the preceding figure. The nucleus of Pander is very poorly developed in this egg. *c.n.*, cleavage nucleus.  $\times 39$ .

<sup>2</sup> In this, as in the succeeding figures of surface views, the anterior margin of the blastodisc is toward the top of the page, and hence the median axis of the later embryo will parallel the sides of the page. In sections the anterior end is always toward the left. In the surface views the area occupied by the primary cleavage, together with the first "ring" of periblast, are shown.



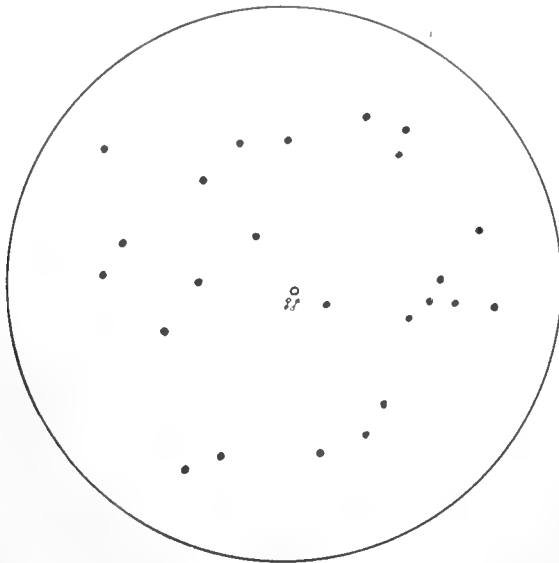
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4

FIG. 3.—Enlarged drawing of the cleavage membrane of the preceding figure. *c.m.*, cleavage membrane; *m.p.*, membrane plate; *v.*, vitelline membrane.  $\times 525$ .

FIG. 4.—Enlarged drawing of the extreme left end of the section shown in Fig. 18. This shows a supernumerary sperm nucleus, *s.p.*, about which cell formation is attempted, as evidenced by the horizontal cleft situated just above the nucleus.  $\times 525$ .



5

FIG. 5.—Diagram of an unsegmented blastodisc showing the distribution of twenty-four sperm nuclei. This egg was removed from the oviduct about two and a half hours after the estimated time of fertilization, when it was eleven inches from the infundibulum. See Table 1, hen No. 1.  $\times 18$ .

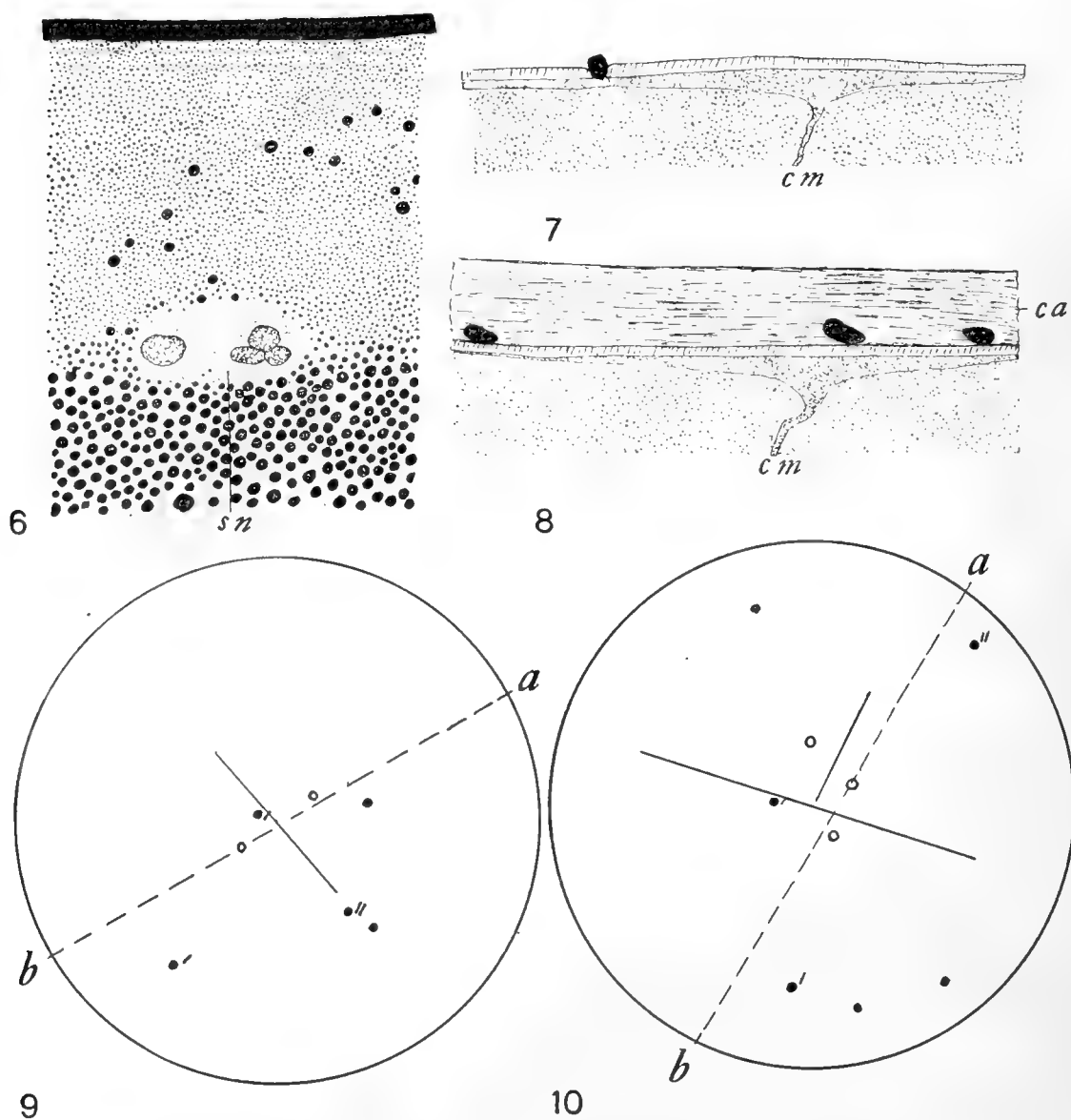


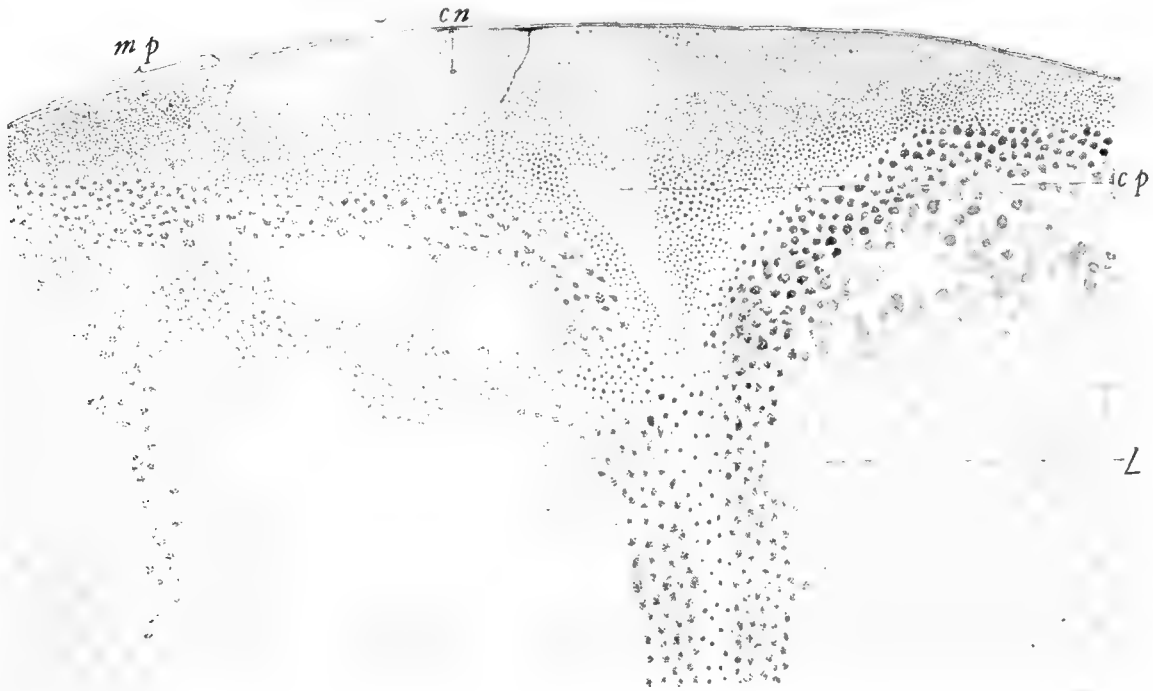
FIG. 6.—A nest of four supernumerary sperm nuclei.  $\times$  about 600.

FIG. 7.—A sperm head lodged in the vitelline membrane. The sperm had evidently been stopped in the act of entering the egg.  $\times$  about 600.

FIG. 8.—Three sperm heads embedded in the chalazipherous layer of albumin and located next to the vitelline membrane. Both of these figures (7 and 8) are taken from the central region of the egg shown in Fig. 10.  $\times$  about 600.

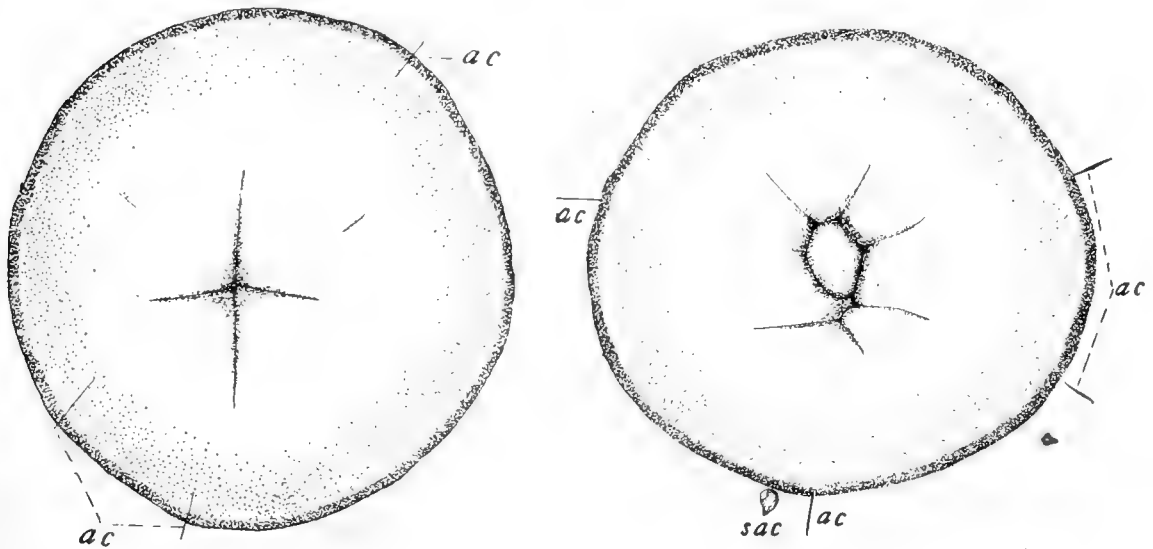
FIG. 9.—A diagram of the blastodisc shown in Fig. 1, showing the distribution of the supernumerary sperm nuclei. The black dot indicates that the nucleus is located more or less superficially in the disc; while a dot marked prime one shows the location of a nucleus that is situated deep in the disc, and one marked prime two, a nucleus that is undergoing fragmentation. The broken line, A—B, is the plane of the section shown in Fig. 2.  $\times$  18.

FIG. 10.—Diagram of a blastodisc of an egg taken from the oviduct three hours after fertilization, shortly after it had entered the isthmus. This shows six supernumerary sperm nuclei. Line A—B is the plane of the section shown in Fig. 11.  $\times$  18.



11

FIG. 11.—A section through line A—B of Fig. 10. *m.p.*, marginal periblast; *c.n.*, cleavage nucleus; *l*, neck of the latebra.  $\times 52$ .



12

13

FIG. 12.—The four-celled stage, drawn from a whole mount preparation. The egg as taken from the isthmus (see Table 1, hen No. 3). *a.c.*, accessory cleavage furrows.  $\times 18$ .

FIG. 13.—An eight-celled stage, drawn from a whole mount preparation. The history of this egg is given in Table 1, hen No. 4. It shows one central and seven marginal cells. *a.c.*, accessory cleavage furrows; *s.a.c.*, small accessory cleavage cells.  $\times 18$ .



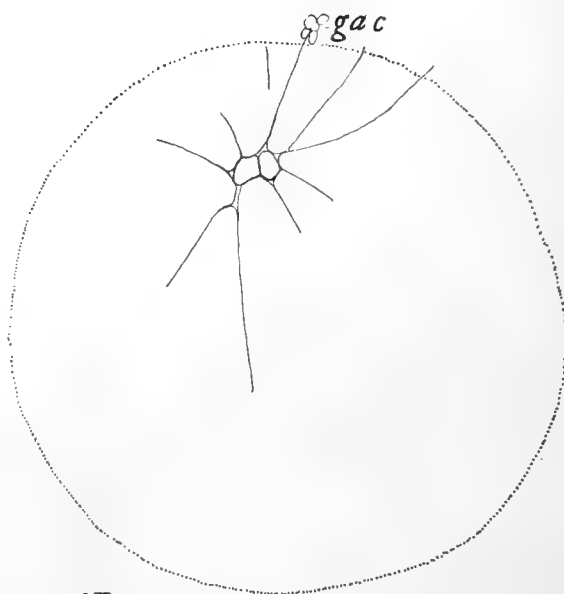
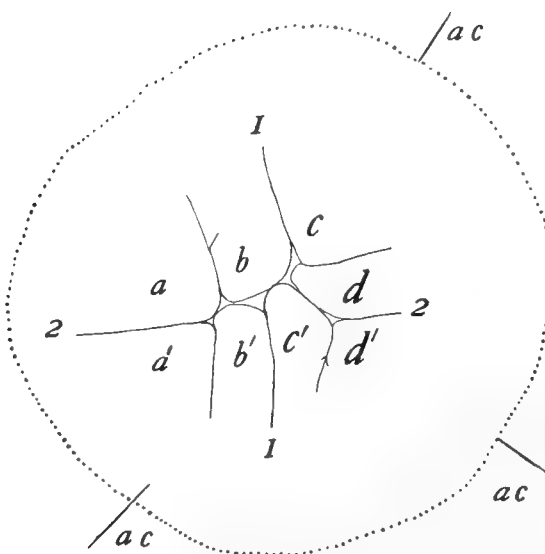
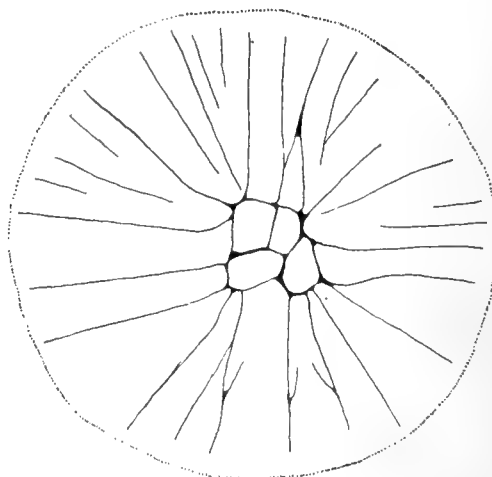
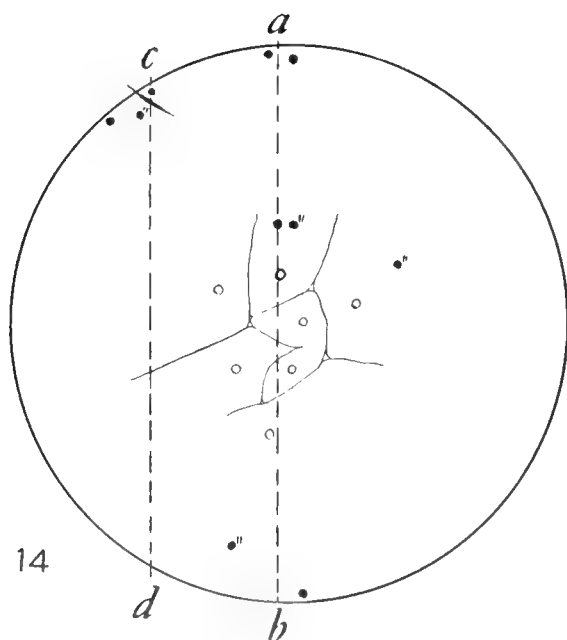
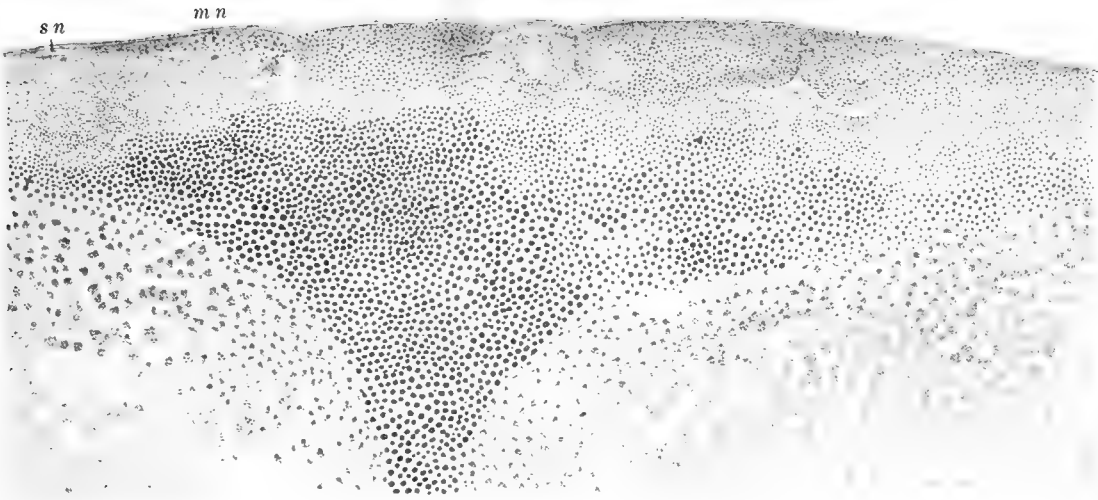


FIG. 14.—A diagram of a seven-celled stage, showing the distribution of the cleavage and supernumerary sperm nuclei. The egg was taken from the isthmus about three and three-fourths hours after the estimated time of fertilization. A—B, plane of the section shown in Fig. 18, and C—D that of Fig. 19.  $\times 18$ .

FIG. 15.—An interesting blastodisc showing a large number of marginal cells in the process of formation. The egg was taken from the isthmus.  $\times 18$ .

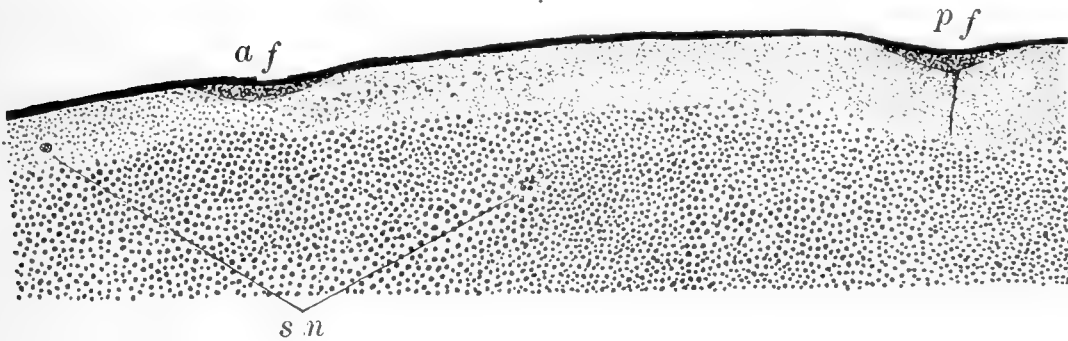
FIG. 16.—A blastodisc showing a comparatively regular form of cleavage in the eight-celled stage. The egg was taken from the isthmus about four hours after fertilization. The letters indicate the cells that are probably homologous; and the numbers, the first and second cleavage planes. Three accessory cleavage furrows are shown.  $\times 18$ .

FIG. 17.—An early stage showing an eccentric cleavage, with the displacement toward the anterior. *g.a.c.*, a group of five small accessory cleavage cells. The egg was taken just as it was passing into the shell-gland.



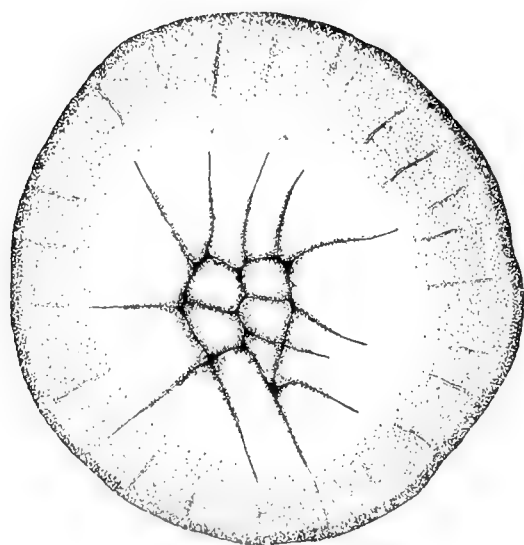
18

FIG. 18.—Section through plane *a—b*, Fig. 14. *m.n.*, marginal cell nucleus; *s.n.*, supernumerary sperm nucleus with a cleft lying just above it.  $\times 73$ .

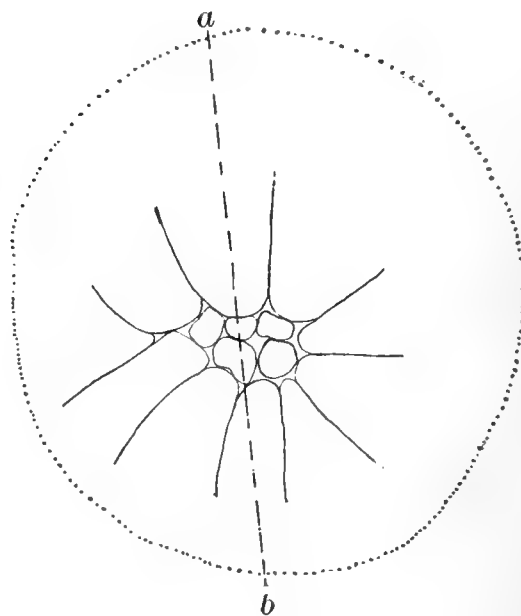


19

FIG. 19.—Anterior portion of a section through *c—d*, Fig. 14. *s.n.*, sperm nucleus; *a.f.*, accessory cleavage furrow; *p.f.*, terminal portion of a primary cleavage furrow with membrane.  $\times 66$ .



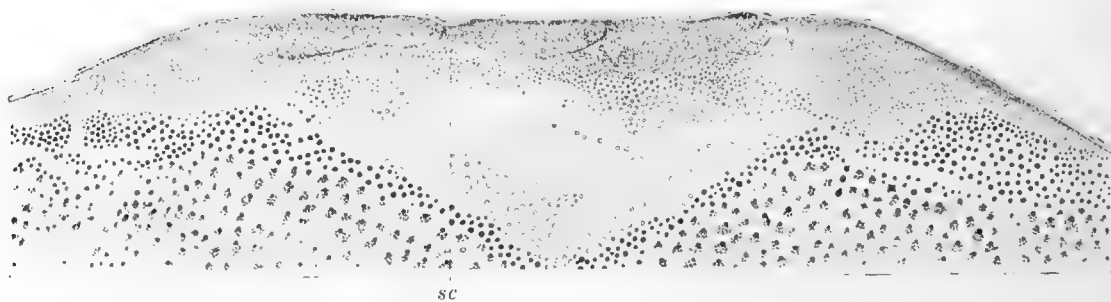
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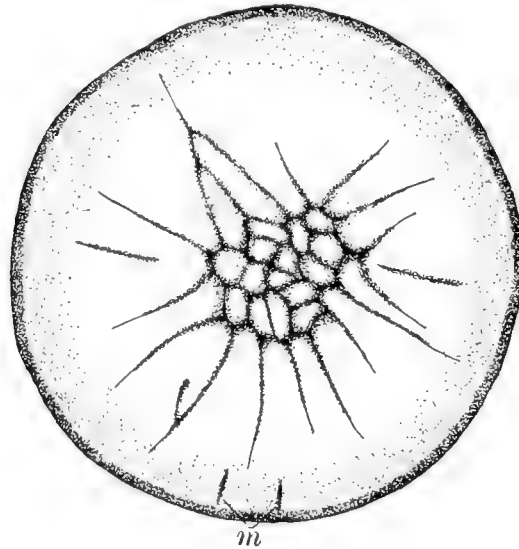
FIG. 20.—A seventeen-celled stage, drawn from a whole mount preparation. The egg was taken from the isthmus between four and five hours after fertilization. The blastoderm is remarkable in that it has many short radial furrows situated near the margin of the primary area (see text for a description of these furrows).  $\times 18$ .

FIG. 21.—A fifteen-celled stage, drawn from the living egg (see Table 1, hen No. 5). *a—b*, plane of section shown in Fig. 22.  $\times 18$ .



22

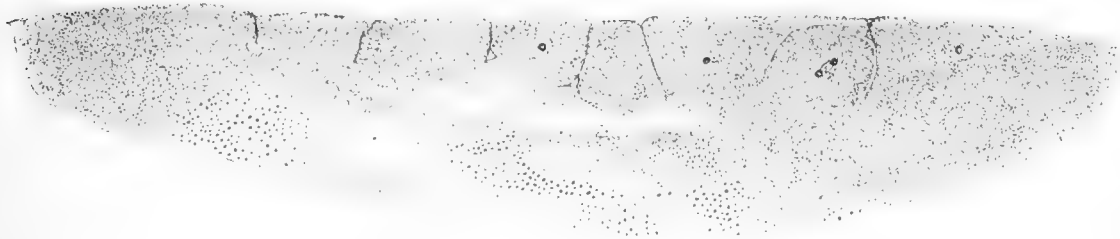
FIG. 22.—Section through plane *a—b*, Fig. 21. *s.c.*, segmentation cavity.  $\times 55.5$ .



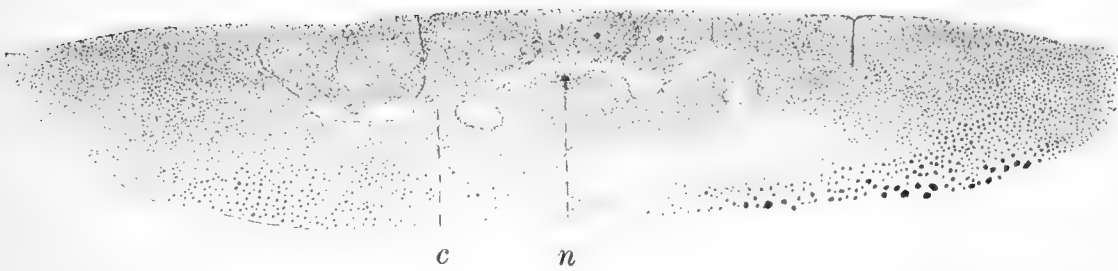
23

FIG. 23.—A thirty-four-celled stage, drawn from a whole mount preparation. The egg was taken from the shell-gland. There are seventeen marginal and seventeen central cells. At the posterior margin are shown two of the short radial furrows which were noted in Fig. 20.  $\times 18$ .

A

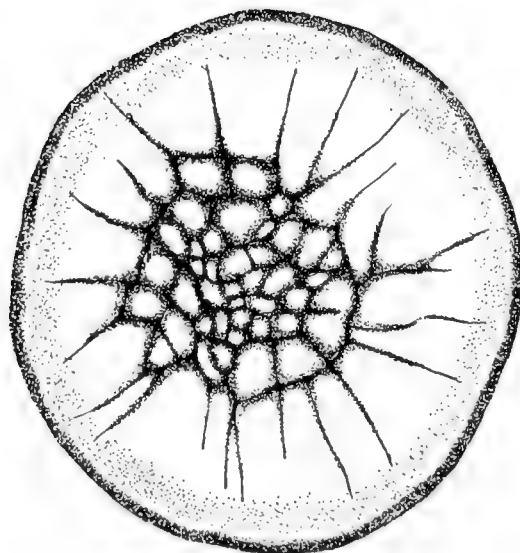


B



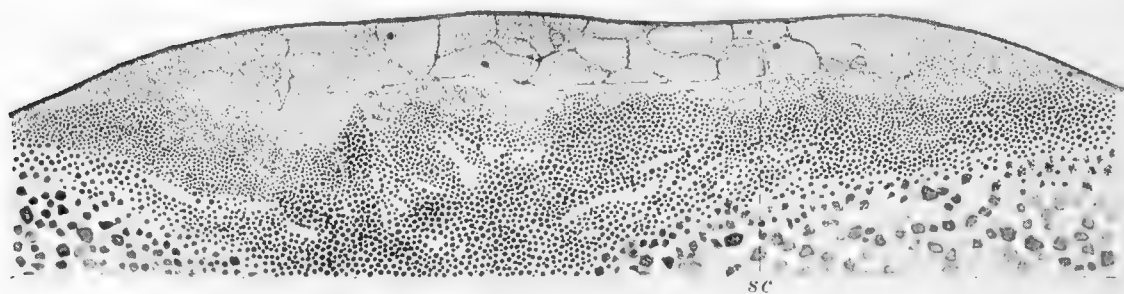
24

FIG. 24.—A. Median section of a blastoderm showing thirty-two cells. B. A section taken seven sections to the left of the preceding. *n*, nucleus; *c*, cell showing a connection with the floor of the segmentation cavity (see text for a description of these figures). Both  $\times 59$ .



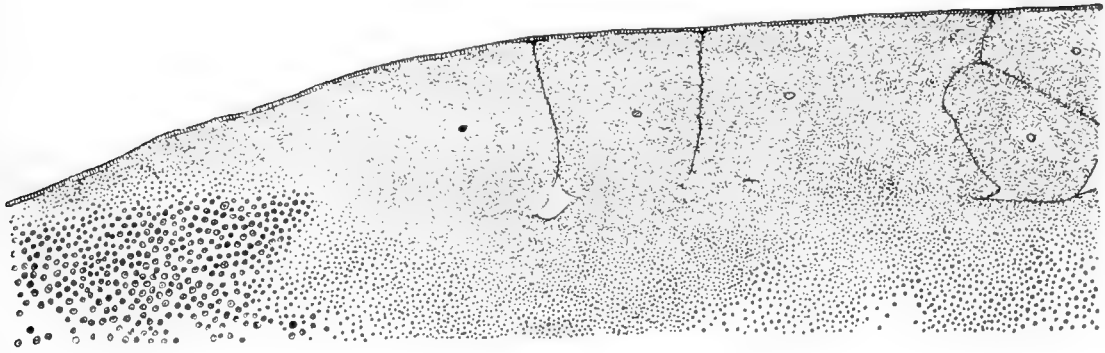
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FIG. 25.—Blastodisc showing sixty-four cells in surface view—forty-one central and twenty-three marginal (see Table 1, hen No. 7).  $\times 18$ .

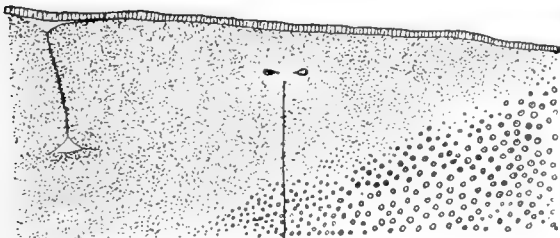


26

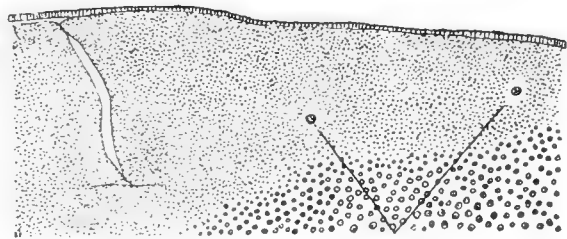
FIG. 26.—Median longitudinal section of a blastoderm in a stage of development corresponding to that of the preceding figure. *s.c.*, segmentation cavity.  $\times 55$ .



27

*m c n*

28

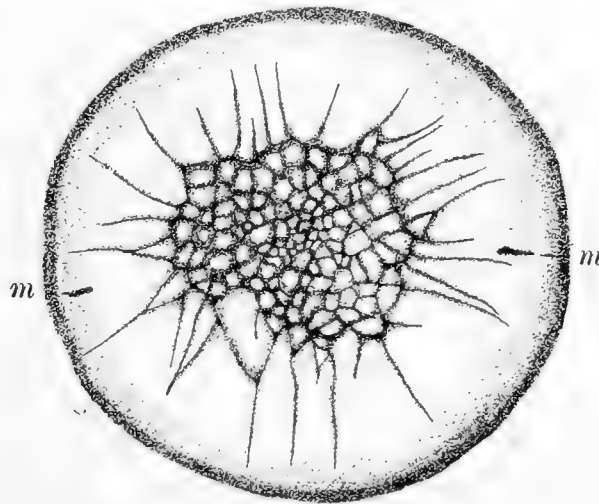
*m c n*

29

FIG. 27.—The anterior end of a section located to the right of the one shown in the preceding figure. This gives the details of structure of the margin of the disc. Some of the nuclei are taken from adjacent sections.  $\times 139$ .

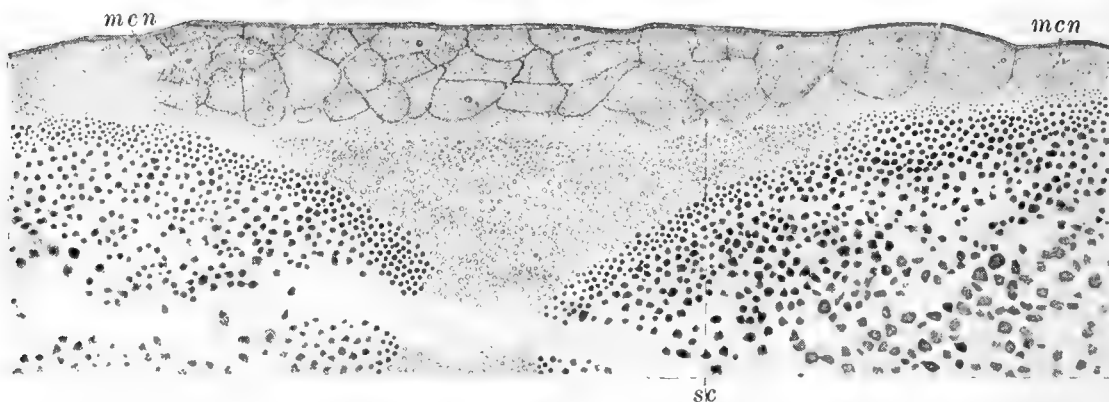
FIG. 28.—Section of a marginal cell from the same series as the section shown in Fig. 31. *m.c.n.*, marginal cell nucleus, undergoing division.  $\times 139$ .

FIG. 29.—Another marginal cell from the same series, showing how the sister nuclei have migrated apart. *m.c.n.*, sister nuclei of the marginal cell.  $\times 139$ .



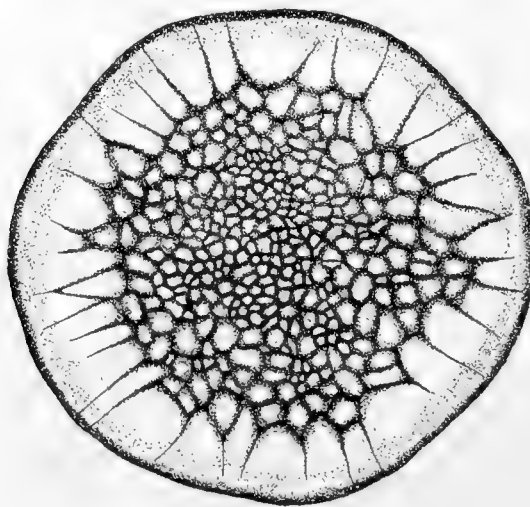
30

FIG. 30.—Surface view of a blastoderm of an egg taken from the shell-gland seven hours after fertilization (see Table 1, hen No. 8). There are 31 marginal and 123 central cells, or a total of 154 in the surface view. *m.*, short radial furrows at the margin.  $\times 18$ .



31

FIG. 31.—Median longitudinal section of a blastoderm in a stage of development corresponding to that shown in Fig. 30. *m.c.n.*, marginal cell nucleus; *s.c.*, segmentation cavity.  $\times 65$ .



32

FIG. 32.—A blastoderm showing 346 cells in the surface view, 34 marginal and 312 central (see Table 1, hen No. 9). Note that the marginal cell furrows are beginning to cut out into the periblast.  $\times 18$ .

# A STUDY IN THE GERM CELLS OF LEPTINOTARSA SIGNATICOLLIS

HARRY LEWIS WIEMAN

*From the Hull Zoölogical Laboratory, University of Chicago*

WITH SEVENTY-THREE FIGURES

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## INTRODUCTION

*Leptinotarsa signaticollis*, a chrysomelid beetle found in the northern and eastern tributary valleys of the Rio Balsas system in Mexico (Tower, '06), is a favorable form for cytological investigation, in as much as the long breeding season, the slow development of the reproductive organs, together with the fact that the insects can be successfully bred and reared in breeding cages, enables one to obtain any stage in development with relative ease.



The present paper embodies a study of important stages occurring for the most part in the pre-maturation period of the germ cells. Recent discoveries in chromosome behavior have brought the reproductive cells of insects into considerable prominence; but most of this work has been prosecuted along rather narrow lines. The chromosomes are undoubtedly important elements, but they are far from being the only factors to be considered in a study of the mechanism of heredity. The cytoplasm, though long neglected, is coming more and more to be recognized as of equal if not greater importance in this regard; and in the present undertaking the so-called nucleo-cytoplasmic relationship has been studied by following the morphological and chemical transformations undergone by the cellular elements of the ovary and testis during critical stages of development.

The ovum offers a more extended field for observation than the spermatozoan; since spermatogenesis is a relatively simple matter compared with the complex changes involved in the production of a mature ovum. As a result I have given more attention to the reproductive organs of the female than to those of the male.

To Professor C. O. Whitman, at whose suggestion this work was taken up, and to Professor W. L. Tower, who kindly supplied me with material from his pedigreed stock, I am greatly indebted for much valuable advice and criticism.

## METHODS

Two distinct methods of killing and fixing were followed depending upon whether chemical or morphological differentiation was the object sought after. Experience showed that Flemming's strong solution was far superior to any other reagent for faithful preservation of morphological detail; but the presence of osmic acid greatly interferes with the action of stains employed in the study of chemical changes. For the latter purpose I used a saturated aqueous solution of picric acid to which sufficient acetic acid was added to make a 10 per cent solution. This does

not give as perfect fixation, but enables one to study chemical transformations more satisfactorily. This mixture was used almost exclusively for eggs in later stages of development. The eggs were allowed to remain for about ten minutes in the reagent which was maintained at a temperature of 55°C., and then transferred to 70 per cent alcohol. At the end of a day or two the egg envelopes stand out from the egg, and can be readily dissected off with the aid of needles. Good results were also obtained with Hermann's platino-aceto-osmium mixture. The killing fluids were used in the cold except in the case of the eggs as noted above.

The most satisfactory stains were found to be safranin (basic), and lichtgrün (acid). Others were used in various combinations, but without such good results. Even for the study of chromosomes I have found these dyes superior to Heidenhain's iron-alum-haematoxylin. Grübler's "Safranin 0" was made up according to the following formula:

Safranin.....	1 gram
Anilin water (4cc. anilin oil + 90 cc. water).....	90 cc.
Alcohol (95 per cent) .....	10 cc.

Sections were left in the safranin for four to six hours, passed through graded alcohols and immersed for a few seconds in the acid stain. After washing in 95 per cent alcohol, the material was transferred successively to absolute alcohol, clove oil and xylol; and then mounted in balsam. These two stains work together perfectly, and give sharp and clear contrast at every stage.

Iron-alum-haematoxylin with or without counterstain (orange G or lichtgrün) and Gram's gentian violet method were also employed, though to a less extent.

For embedding, Johnston's paraffin-asphalt-rubber mixture was used in various degrees of dilution with pure paraffin. This method is especially helpful in working with mature eggs, which show a great tendency to crumble when cut in pure paraffin. Sections were as a rule cut  $6\frac{2}{3}\mu$  in thickness.

## 1. THE DEVELOPMENT OF THE OVARY

*The cell elements of the ovary*

The insect ovary has long been a favorite object for microscopical study, yet some of the most important aspects of the problem it presents are unsatisfactorily answered. The organ exists in a wide variety of morphological types, but however great its lack of constancy in macroscopical structure, it always shows in the egg-tube the presence of three elements: germ cells, nurse cells and epithelial cells. One of the questions that has engaged the attention of many investigators is the origin of these cells.

Following the contributions of Dufour ('33, '41) and others on the gross anatomy of the insect ovary, Stein ('47), in his monograph on the female genital organs of a large number of Coleoptera, published the results of the first thorough investigation of the histology of the ovary, and thus laid the foundation for all subsequent work in this field. He showed that the terminal thread (*Endfaden*) is not a blood vessel as had been stated years before by Johannes Müller ('25), but that in all probability it serves as a suspensory ligament which binds the ovarioles together and fixes them to the dorsal wall of the thorax. The eggs, he believed, arise from the large cells in the lower or proximal part of the terminal chamber, and that the cells in the other part of the tube are the yolk-building elements, *i.e.*, nurse cells. He correctly described the two sheaths of the ovary, the outer "*peritoneal Hülle*" and the inner structureless "*tunica propria*." To the germinal vesicle he attributed the morphological value of a cell, and judging from more recent work, he also erred in considering the chorion of the egg a result of the fusion of follicle cells, instead of a secretion product of these cells, as later research has demonstrated.

H. Meyer ('49) described two kinds of so-called "*nuclei*" in the ovary of *Lepidoptera*, small ones of an epithelial nature, and large ones which develop into germinal vesicles, while the nurse cells were regarded as aborted ova. Here then is the first claim for a common origin for reproductive and nurse cells, the epithelial cells having a different origin.

During the next decade or more, most investigators were occupied with the study of the structure and development of the various envelopes of the egg and ovary, but in 1864, Claus, returning to the question of the origin and significance of the three recognized cell elements, came to the conclusion that they were all of common origin, being derived from the primordial germ cells. This conclusion was shortly afterward confirmed by Leuckart ('65) and Landios ('67), but Leydig ('66), on the other hand, questioned this result, and held that only egg and nurse cells are "in ihrer Wurzel identisch," and "Epithel hingegen, besteht für sich, und es findet kein Uebergang zu dem Keim-und Ei-Zellen statt." Likewise, Metschnikoff ('66), in his investigation of the embryonic development of *Cecidomyia* arrived at a similar conclusion namely: that the egg and nurse cells are derived from the "Polzellen der Geschlechtsorganen," while the epithelial cells have an entirely different history.

From a study of *Nepa* and *Notonecta*, Will ('85), described an entirely new as well as unique method of egg formation, in which both nurse and germ cells arise inside of the large "*nuclei*" filling the terminal chamber of young insects, and which he called "*Oöblasten*." Later, the rupture of the membrane permits the contents of the nuclei to pass out, when the remaining part of the oöblast reconstructs a membrane and becomes a germinal vesicle. Similar processes have been described by Sabattier ('86) and Perez ('86). The latter states that three kinds of nuclei, representing egg, nurse, and epithelial cells, are at first enclosed in a mother cell.

Will's theory has been severely criticized by Korschelt ('86), who has shown a different origin for these cells in a large number of species. Korschelt supports Claus's idea of a common origin for all three kinds of cells.

However, Leydig ('89) again produced evidence of a separate origin for germ and epithelial cells, and this view has in latter years been steadily gaining ground. Thus Heymons ('91) showed that in *Phyllodromia* (*Blatta*) *germanica*, the primitive germ cells appear even before the somites are established, while the cells of the terminal thread and the epithelial cells are derived from the

dorsal wall of the mesoblastic somites. Later, Heymons ('95), extended his work to the *Dermaptera* and *Orthoptera*, and confirmed this conclusion. Wheeler ('93) in *Xiphidium ensifermis* was unable to detect the germ cells until the somites are formed when they appear as metameric cell clusters, each of which is confined to the median portion of the splanchnic wall of the somite. These two authors do not agree as to the exact time of appearance of the primordial germ cells, but they show very clearly that certain cells, differing from the ordinary mesodermal or epithelial cells are established at an early period in ontogeny, and constitute the material from which the germ cells arise. Carrière und Bürger ('98), came to similar conclusions for *Chalicodoma muraria*.

In a study of the embryology of *Donacia crassipes*, Hirschler ('09) states, "die Geschlechtsanlage bei *Donacia* schon vor der Entwicklung desselben an der ganzen Eioberfläche als histologisch differenzierte Zellenanhäufungen auftritt. Die Genitalzellen sind also ontogenetisch älter als die Keimbätter" (p. 637). More recently, Hegner ('09), published the first connected account of the Keimbahn in insects, which established beyond a doubt the early differentiation of the germ cells, thus excluding the possibility of the presence in the egg-tube of indifferent cells which might give rise to either epithelial or sexual cells.

Between these early embryonic stages and the adult condition, there is a wide gap in our knowledge of the developmental process. Fairly extensive comparative studies based on adult and slightly younger stages have been made (Gross ('03), Kohler ('07) etc.), but in order to study the history of a continuous developmental process such as the differentiation of the elements of the ovary, it is necessary to have a complete series of normal stages taken from a single type. One can not tell from an examination, however intensive, of the completed adult structure, how that structure has been brought about. Inspection of a large number of sections taken from later developmental stages, especially larval and pupal, indicated to me that a study of this period of the formation of the ovary would yield interesting and important data, and throw some light on the histology of the adult organ.

The adult female reproductive organs of *L. Signaticollis* con-

sist of a median vagina with a somewhat funnel-shaped oviduct on either side. To the broad distal ends of each oviduct are attached from 45 to 46 ovarioles or egg tubes, which are of a type common to a large number of Coleoptera. Each tube is divided into three more or less distinct regions, namely; the ovariole stalk (*Rohrstiehl*), which is the part proximal to the oviduct, the terminal chamber (*Endkammer*), in the lower part of which the egg passes through its early development, and the terminal thread (*Endfaden*), which is dilated at its base into a broad cap-like

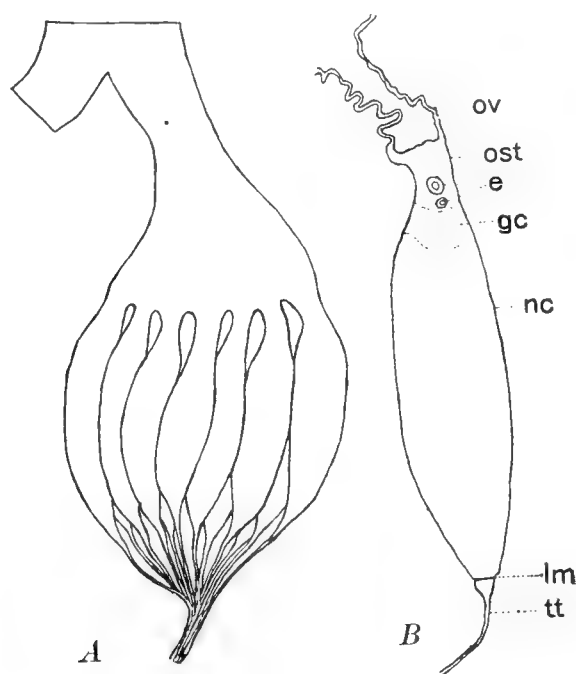


FIG. W. A, ovary; B, single ovariole represented in longitudinal section; e, egg; g. c., germ cells (young ovocytes); l. m., limiting membrane; n. c., nurse cells; o. st., ovariole stalk; ov., oviduct; t. t., terminal thread.

structure marked off from the terminal chamber by a definite membrane (Fig. W.). In the terminal chamber are found three different kinds of cells, the egg cells in the lower proximal region, the nurse cells occupying the distal part ( $\frac{7}{8}$ ) of the chamber, and the epithelial cells scattered between those of the other two groups.

In seeking for a stage at which to begin this account, I found the close of the larval period to be the most advantageous, for here very simple conditions prevail which permit one to distinguish,

without the slightest doubt, between germ and epithelial cells; while the nurse cells have not yet been differentiated. Furthermore, the germ cells are practically unaltered in appearance from early embryonic stages. Figure 1, represents a longitudinal section of an egg tube characteristic of the larval condition, in which one of the striking features is the direct continuity between the cells of the terminal thread (*tt*) and those of the terminal chamber. In the region of the terminal chamber two kinds of cells can be readily made out, the large germ cells (*g.c.*) with deeply staining cytoplasm and with nuclei showing an irregular chromatin reticulum, and the small pale epithelial cells (*ep.c.*) continuing outward into the terminal thread. The ovariole stalk is composed of tall columnar cells (*o.st.c.*). The peritoneal sheath is in the process of formation, and is represented by the large epithelial cells (*p.sh.*) which are flattened against the sides of the tube. Beneath this outer sheath lies the tunica propria (*t.pr.*) bounding the terminal chamber and thread. The ovarioles are united by their terminal threads coming together on either side into a single bundle which is inserted into the dorsal wall of the body cavity. (Fig. W, A).

The epithelial cells scattered between the germ cells are what the older authors designated as the small nuclei surrounding the ovocyte, to which they supply nutrition. In the figures of these authors, these cells are never shown with cell boundaries, but always as nuclei lying in a homogeneous matrix. At best it is not easy to make out cell boundaries, since the cytoplasm shows such a weak affinity for the stain, but a careful study of many sections at different stages has convinced me that cell walls are present and that each nucleus belongs to a single cell, except under certain conditions as noted below. At *a.c.* is a large cell which I have designated the *apical cell*, the significance of which I have been unable to determine. It is also seen in later stages (Figs. 2,3).

The fact that the epithelial cells show such a slight affinity for the stain, seems rather strange in view of what appears to be their function; namely, to supply nourishment to the germ cells. Ordinarily, cells having a secretory function show in their cytoplasm more or less granular masses which stain deeply. Are they

really nutritive in function? The evidence for believing so rests on their close relationship to the germ cells, and on the changes undergone in size and form after coming into contact with the germ cells. It seems to be the general concensus of opinion that these cells later form the follicle of the egg by surrounding the latter as it passes from the lower region of the terminal chamber; but I believe it can be readily shown that the follicle is not formed in this manner. The evidence for this will be given in its place a little later.

Closer examination of the germ cells in the stage shown in Fig. 1, reveals the presence of one or two basic-staining nucleoli, each surrounded by a clear non-staining area (Fig. 34). This condition proves to be the result of the division of a single nucleolus, and this is the first step in what appears to be a process of amitosis. The division later extends to the nucleus as shown in Fig. 35. A fuller discussion of this process and its significance will be considered in connection with the germ cells of the male.

In Fig. 2, which is from a very young pupa, we find a considerable increase in the number of cells in the ovariole, brought about by mitotic divisions which are very abundant at this and succeeding stages, especially among the germ cells. Mitotic figures among the epithelial cells are very rare, but as they do occur, it is inferred that mitosis is the method of cell multiplication, for I have never observed authentic cases of amitosis among these cells at this stage. The region of the terminal thread is much larger in diameter than in Fig. 1. The cells at the base of the thread show a tendency to flatten out, and the lower margin of these flattened cells represents the position of the future limiting membrane which will definitely separate the terminal chamber from the terminal thread.

Fig. 3 is from a slightly older pupa, and shows at *l.m.* more distinctly the place where the limiting membrane will form. Just below this is seen a double row of epithelial cells, which compared with those surrounding the germ cells lower down, show considerable differences in form and size. The latter are smaller, and the nuclei are bean-shaped. These transformations suggest that these cells are in some way concerned with the metabolism of



the germ cells. However, as will be shown later, the accumulation of these cells at the lower end of the terminal chamber seems to be involved in the differentiation of the germ cells, and it may be therefore, that these form changes are simply due to mechanical pressure as the cells make their way down the tube. Fig. 4 is a cross section of an ovariole showing the same relationship.

During the next few days the terminal thread undergoes a remarkable growth, as a result of which its diameter is increased enormously. In Fig. 5 is seen a longitudinal section of it at maximum development, when it greatly exceeds the terminal chamber in volume. The same figure shows another stage in the formation of the peritoneal sheath, which for the sake of simplicity was omitted from several of the preceding drawings. Fig. 10 is from a pupa about two days older, in which it can be readily noted that the terminal thread has diminished very considerably in volume. The process continues until in the adult it has the appearance shown in Fig. 13, where throughout the greater part of its length there is evidence of degeneration, leaving at its base a cap-like mass of cells which is practically all that remains of the structure shown in Fig. 5.

Fig. 10 shows at the base of the terminal chamber the first appearance of the limiting membrane, which comes into existence first at the periphery, so that the center is the last part to be closed off. The principle evidence for this is the fact, that for a considerable time after it can be definitely made out at the sides, groups of epithelial cells can be seen in the central region entering the terminal chamber. The membrane does not show any cellular structure, and is probably a product of secretion of the epithelial cells at the base of the terminal thread.

Wagner ('36, '37), Siebold ('71), Will ('85), and others early opposed the idea of the terminal thread functioning in a purely mechanical manner as a suspensory ligament for the ovariole, and maintained that it is the place where the "Keimstätte" are produced, which in the lower part of the egg tube surround the germ cells. Brandt ('78) thought this interpretation might apply in forms where there was direct continuity between the terminal chamber and thread, but where the two regions are separated by

a limiting membrane, he believed the terminal thread served as a ligamental structure. Kramer ('69) regarded the thread as the solid continuation of the *tunica propria* of the egg tube.

As a matter of fact an almost unlimited variety of functions, from that of a blood vessel to that of a piece of connective tissue, have been ascribed to the terminal portion of the ovariole. This is largely because most workers have confined their studies to the adult organ which represents the end-product of a developmental process. Furthermore, this structure does not present the same appearance in all species, and all gradations are to be found from a condition where it is a mere rudimentary appendage of the egg tube, to cases where it is a direct continuation of the terminal chamber. Agreement as to its significance can scarcely be expected as long as observations are based almost entirely upon the adult structure.

Heymons ('91) was perhaps the first to study the development of the terminal thread, and he showed in *B. germanica* that its cells, as well as the epithelial cells, are derived from the dorsal wall of the primitive mesoblastic somite. According to this author the terminal thread does not contribute epithelial cells to the terminal chamber. In the adult he found the thread ending freely, so that if a supporting function is to be attributed to any part of the apparatus it is concerned with the peritoneal sheath, and not with the cells filling the interior. However, he considers this of no great importance, since the fat-bodies, connective tissue, and tracheal tubes serve to hold the organs in place. The end threads are of functional significance only in embryonic and larval periods of development, when they are concerned with orientation of the ovarioles which, as I understand him, consists in directing the growth of the egg tubes backward and upward toward the dorsal wall of the primitive segments.

The appearance of the terminal thread in the adult of *L. signaticollis* would justify the conclusion that it is a rudimentary appendage of the terminal thread from which it is separated by a definite membrane (Fig. 13 *l. m.*). The peritoneal sheath has become invested with striated muscular tissue and air tracheæ, which form a thick covering grading off into the region of the egg

chamber, into a thin epithelial layer closely applied to the tunica propria. Everything indicates that the peritoneal sheath with its muscular investment, which is inserted into the dorsal body wall, serves to support the ovarioles.

However vestigial its structure in the adult, in the larval and pupal periods the terminal thread is of considerable importance in producing the epithelial cells of the end chamber that later play an important part in the development of the egg. Whether or not it has, during the larval and pupal periods, the function ascribed to it by Heymons, I am not prepared to say, since this is a point that can not be readily demonstrated one way or the other.

It will be noticed in Figs. 5 and 38, that the chromatin of the germ cells has a peculiar granular appearance that is quite different from the reticular structure of the nuclei of the germ cells at the lower end of the tube (Fig. 6). This is the first indication of the differentiation of the germ cells into functional sexual cells that will develop into eggs, and nurse cells.

The question immediately arises as to what causes this differentiation. Giardina ('01) described in *Dytiscus marginalis* a process of differentiation in which one of the daughter nuclei, resulting from the division of a primordial germ cell, receives in addition to the usual number of chromosomes, a certain amount of chromatin, and develops into a functional germ cell; whereas, the other daughter cell lacking this extra chromatin becomes a nurse cell. Nothing of this kind is to be observed in *L. signaticollis*.

At just about this time in the developmental history, there occurs at the junction of the terminal chamber and tube stalk a transformation that is significant in this regard. Fig. 6 shows the condition immediately preceding the change; the boundary between the egg chamber and the tube stalk is sharp, and the cells of the latter can be distinguished from the epithelial cells not only by their shape, but by the capacity of the cytoplasm to stain more deeply. Fig. 7, which is a few days older, shows a pale, lightly staining, semi-fluid mass that blends with the epithelial cells on the one hand and the cells of the ovariole stalk on the other. It is very difficult, impossible in some cases, to make out cell bound-

aries, which suggests that a process of liquefaction is taking place, as a result of which the walls of the epithelial cells and to a less extent those of the stalk cells are dissolved, producing a matrix in which the nuclei of these cells are suspended.

This diffuse effect is not a chance occurrence to be observed in a few odd preparations, a result which might be attributed to an artifact, but is an event that takes place regularly at what appears to be a critical period in the development of the ovum.

As this condition comes on at the time when the chromatin configuration enables one to distinguish between egg cells and nurse cells, it seems that there might be some causal relation between the two phenomena. A study of the epithelial cells demonstrates that they filter down from the upper portion of the terminal chamber to its lower end, where they accumulate, and the result is an interaction between them and the contiguous cells of the ovariole stalk. The semi-fluid matrix formed might then be regarded as exerting a specific effect on those germ cells coming under its influence, enabling them to develop into ova, while the more distant germ cells become nurse cells.

For some time preceding this stage the germ cells multiply so rapidly that the daughter cells do not accumulate much cytoplasm; and a section at this time shows the cells as large clear nuclei, containing chromatin in the form of a spireme, around which is a very narrow margin of cytoplasm (Fig. 8). These cells have completed their division period and therefore represent the last generation of ovogonia. As they enter upon the growth period they move down into the diffuse region. Fig. 9 is from an adult several days older, when the eggs appear with a considerable quantity of cytoplasm which increases steadily in amount from now on.

It is also to be noted that mitotic divisions continue in other parts of the egg tube long after they have ceased in this region. This fact tempts the suggestion that the energy which is preserved in the ovocytes for the future maturation divisions and perhaps the development of the embryo, is expended in mitotic divisions by the nurse cells, the sister cells of the ovocytes. The occurrence of tripolar spindles (Fig. 19) and abnormally large single spindles that result in the production of gigantic cells, are manifestations

of irregular cell activity among the future nurse cells that lend additional support to the idea.

Do the egg cells cease to multiply because they have passed through a fixed number of cell generations; or does the cause lie in the action of some substance produced in the egg tube that prevents further cell division, and turns the direction of cell activity into different channels, the result of which is growth? Much evidence favors the latter view, for we are learning more and more that development and differentiation are largely a matter of correlation of mechanical and chemical forces. Thus Spemann ('01), Lewis ('04), Le Cron ('07), and others have shown that the development of the lens of the eye in certain amphibia depends upon a stimulus set up in the ectoderm in the region where the outgrowing optic vesicle touches it. In what is known as "hormone action" we have an example of the secretion of one organ reacting upon another organ in such a manner as to cause secretion in the latter, or to affect its metabolism in other ways. The phenomena accompanying the oncoming of puberty and the results of castration are too well known to need mention.

Such facts indicate that the interaction, whether by mechanical contact or through the production of chemical combinations of tissues or their secretions upon each other is an important factor in developmental processes. Therefore, I believe this semi-liquid condition occurring in the terminal chamber at a definite time in the history of the organ, is to be regarded as a physical and chemical reaction in which the epithelial cells of the egg chamber and those of the ovariole stalk are involved, and that it is an important, if not the causative, factor in the differentiation of the primordial germ cells into egg cells and nurse cells.

From this it follows that the epithelial cells, which come chiefly from the terminal thread, are very intimately connected with the development of the germ cells, but I do not believe they take any part in forming the follicles of the egg. These are formed from the columnar cells of the tube stalk. In the first place, the epithelial cells are never found below the lower limit of the diffuse area, while the follicles are always formed below this level (Fig. 11). In the second place, the follicle cells (Figs. 11, 12) bear an

unmistakable resemblance to those of the stalk in early stages (Figs. 1, 2), whereas the epithelial cells are of a totally different appearance.

Both kinds of cells have their origin in the mesodermal somites (Heymons '91, Wheeler '93), but the cells of the ovariole stalk early undergo a differentiation which distinguishes them from the epithelial cells. The former are columnar in outline and stain deeply, while the latter are round or oval and show no affinity for the stain.

In the differentiation of the germ cells into egg and nurse cells, we have a very good example of the general conclusion which Whitman ('93), F. R. Lillie ('06), and others have reached; that morphogenic processes can not be conceived as merely the sum total or the resultant of the individual cell activities, but that the organism (the ovary in the present case) operates as a unit without respect to cell boundaries. There is no reason for believing that the primordial germ cells differ in nuclear contents; neither is there any evidence of a differentiating division of the chromatin which would predetermine which of them were to become functional ova and which nurse cells. On the contrary, the process seems to be the result of the activity of several distinct cell elements which operate together as a whole.

### *The Formation of the Egg Strings.*

The development of the egg string was first studied by Leydig ('66), who regarded it as a portion of the protoplasm of the egg. Its relation to the nurse cells does not seem to have been very clear, especially in telotrophic ovarioles such as are present in *L. signaticollis*. For a long time it was supposed that the eggs of these forms were without egg strings and that the follicle cells supplied the ovum with nutrition. Will ('85) gives a diagram (Fig. 11) showing the relation of the strings to the eggs in *Nepa* and *Notonecta* that portrays the condition in my material, but he says nothing about their formation or the nature of their connection with the nurse cells.

In *L. signaticollis*, at the appearance of the semi-fluid condition described at the junction of the egg tube with its stalk, the germ cells exhibit signs of amoeboid movement and move away from each other; the first step in the process being shown in Fig. 9. The young eggs are just entering the growth period, and are taking positions in a linear series. As they separate they leave behind a strand of protoplasm which comes into relationship with the nurse cells through the medium of the matrix. Fig. 11 is a rather fortunate section showing a number of egg strings in various degrees of development. It should perhaps be stated that in Fig. 11 the distal end of the ovariole is toward the bottom of the page, whereas in Figs. 6, 7, 8, and 9 the distal end is toward the top of the page.

These nutritive strings are very delicate structures composed of thin strands of the cytoplasm of the egg, drawn out like pseudopodia, and owing to their transparency, are very easily overlooked. At the points where they connect with the nurse cells, the strings blend with the intercellular region.

The nurse cells as shown in Fig. 11, are large polynuclear cells, between which the egg strings terminate. The groove-like spaces between the nurse cells can be compared to ducts into which the nutritive material from the nurse cells is secreted, and from which it is taken by the egg strings into the egg.

As the eggs pass from the semi-fluid region they come into contact with the columnar cells of the tube stalk which form the follicles. After the eggs have taken up their positions, one behind the other in their respective follicles, we find that each string leaves its egg laterally and finds its way back through the follicle cells to the nurse cells (Fig. 12). This figure shows the egg string at the height of its development. Later it disappears and no trace of it can be found in the mature egg.

The real growth period of the egg is initiated with the formation of the nutritive string, and shortly afterward the egg moves down into the tube stalk probably by amoeboid movement or peristaltic action of the ovariole, or both. Korschelt ('86) observed peristaltic movement when the egg tube of *Dytiscus marginalis* was placed in physiological salt solution. I have examined fresh ovaries



of *Leptinotarsa* dissected out in saline solution, but have been unable to detect peristalsis. If it does occur, the action is so slow that it is not readily perceptible to the eye. The irregular outline of the egg at this time points to amoeboid movement as the motive force, although fresh preparations revealed no such movement, but here again it may be so slow as to elude detection.

### *The Nutrition of the Egg*

At the end of the division period (Fig. 8), the ovogonium is nearly all nucleus: the cytoplasm being very small in amount, and pale and almost transparent. With the beginning of the growth process, the cytoplasm undergoes a complete change, taking on a granular appearance and staining deeply. After safranin and lichtgrün, a purplish tint is produced, which is due to the combined color effect of a green acid staining reticulum, through which are scattered more or less uniformly small red granules taking the basic stain. From this point the egg increases rapidly in size, and shows no striking changes in the cytoplasm until it reaches the stage shown in Fig. 11.

The nuclear contents, in the meantime, have undergone considerable transformation. The chromatin passes from the spireme stage of Figs. 9, 41, 42, into a delicate irregular thread staining with safranin (Fig. 43), which gradually loses its sharp outline and its affinity for the basic dye, until finally (Fig. 44) it becomes a filmy, feathery, green staining mass, of irregular outline. At the same time a number of rounded basic staining nucleoli make their appearance.

While the above changes have been taking place a steady stream of nutritive material has been pouring into the egg by way of the egg string. I agree with Köhler ('07) where he states (p. 378) that: "Die Follikel-epithelzellen leisten keinen Betrag zur Ernährung der Oöcyte, dagegen liegt ihnen die Produktion des Chorionbildung materials ob." This conclusion that the follicle cells are concerned with the production of the egg envelopes is in keeping with the view of their origin from the cells of the ovariole stalk, since the latter from the very beginning show in their



deeply staining cytoplasm the evidence of a secretory function that is entirely absent in the epithelial cells. This view of the origin of the follicles is different from that prevailing in the literature, but I am inclined to believe that a similar method of follicle formation may be found to be of much wider occurrence in other species of insects than is at present supposed.

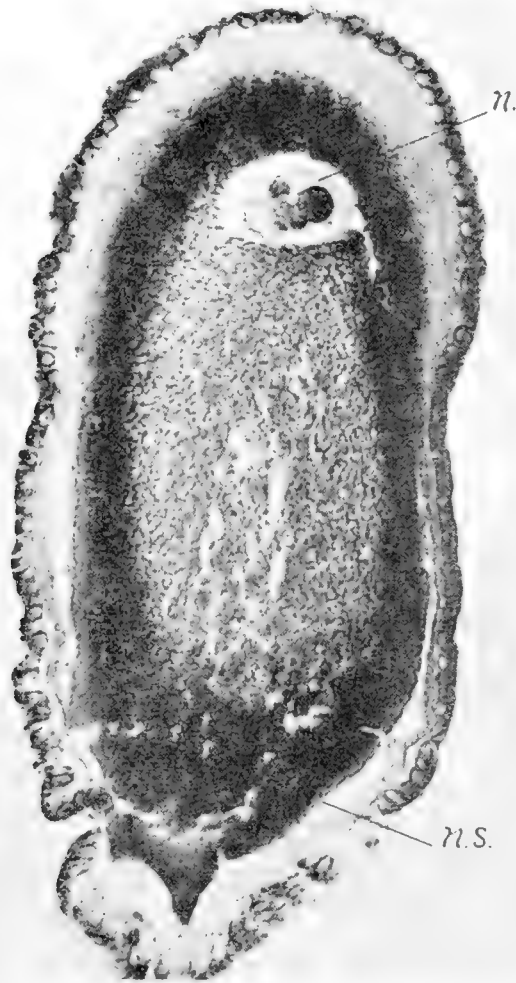


FIG. X. Photograph of a longitudinal section of a half-mature ovum showing the form of the nutritive stream. *n*, nucleus; *n. s.*, nutritive stream.

The red granules appearing with the beginning of the growth period, enter the egg by way of the egg string, and are at first evenly distributed throughout the cytoplasm. As the egg string increases in size, the granules are supplied in larger amounts than can be disposed of, and the result is an accumulation of

the nutritive material extending from the mouth of the nutritive string to the nucleus and even slightly beyond, as shown in Fig. 11. This is from a preparation killed with Flemming's solution, and owing to this method of fixation, the food stream is not differentiated as clearly as in material prepared in other ways. The fact that the nucleus is at this time, composed largely of an acid-staining ground substance, while the granules of the food stream are basic-staining, points to the existence of a chemical attraction between them.

Preparations made with picro-acetic acid, and stained with safranin and lichtgrün were found to be very valuable in studying the nutritive process, and the following account is based on such material.

Fig. X represents a longitudinal section of an egg at the height of the functional activity of the nutritive string. The dark area shows the region where the red basic-staining granules are thickest, and the lighter area where the acid-staining material predominates. The configuration is quite different from that described as typical of the early growth period.

The nucleus (*n.*) shows a green acid-staining ground work in which are embedded a number of more or less vacuolated basic-staining nucleoli of various sizes. The egg string (*n. s.*) is seen leaving the egg at the lower end of the follicle, only a short portion of it showing because of a bend in the tube. This figure shows clearly that the nutritive material is a basic-staining substance; that is, a compound containing an organic acid resembling the nucleic acid of the nucleus in its ability to unite with the dye. Nucleo-albumens have been known for a long time to occur in the yolk of eggs, so that it is very likely that the acid constituent of the nutritive stream is nucleic acid in one form or another. As might be expected this material shows important differences in staining reaction from the chromosomes, since the latter, if they are to be considered identical with the contents of the heads of spermatozoa, yield only phosphoric acid and xanthin bodies as splitting products, while the nucleo-albumens (pseudonucleins) yield protein and phosphoric acid, but no xanthin bodies. Thus while the chromosomes always stain deeply with safranin regard-

less of the killing agent employed, the food stream does not show the same constancy in behaviour. After Flemming's solution it is almost impossible to demonstrate the nature of the food stream by means of basic dyes. More satisfactory results are obtained after picro-acetic acid, but even with this, the food stream can be made to take the acid stain. Similar staining reactions have been observed by many workers in the case of other nucleins, and Mann ('02), p. 339, in his criticism of A. Fischer's views, gives a very good explanation of this behaviour. "When therefore, Fischer observes nucleins to stain readily with basic dyes and only after some delay with acid dyes, it means that the basophil an-ion, nucleic acid, has its basic tendencies incompletely satisfied by the kat-ion albumen radical, and for this reason it readily absorbs some more kat-ions, namely the color base. Conversely, if the nucleic acid is not sufficient to satisfy the demand of the kat-ion albumen, as is the case in those compounds which contain only a little nucleic acid, then some more an-ions are attracted, namely the color acid an-ions." The variations in staining reaction therefore, are not to be taken as indicative of a purely physical or rather mechanical, as opposed to a chemical union between the dye and the substance dyed.

The nutritive stream has undergone considerable change in form since the preceding stage (Fig. 11). On entering the egg (Fig. X) it now divides so as to enclose a more or less pear-shaped portion containing the nucleus, leaving a narrow free margin at the periphery of the egg. The granules are therefore distributed in the form of an oval shell enclosing the yolk which has begun to form in the center of the egg.

Closer inspection of the cytoplasm shows a green-staining reticulum having much coarser meshes than in earlier stages and interspersed with granules from the food stream. In the photograph the lighter areas show the regions where the reticulum is practically free of granules. Fig. 33 represents a section where the two zones adjoin. The food stuff spreads out along the lines of the reticulum toward the center (to the left in the figure) and the periphery (to the right) of the egg in the process of yolk formation. In the course of this process, the granules disappear

and the large polygonal masses of acid-staining yolk are produced in the meshes of the reticulum. In the mature egg, the reticulum is represented by the cytoplasm of the interdeutoplasmic spaces.

This reticular structure may not be present as such in the living egg, but granting that it is an artifact resulting from re-agents used in fixing, the structure is one that varies in size and form at different periods in the development of the egg; and thus may be regarded as representing regions of varying chemical or physical consistency in the living ovum, that indicate the paths of distribution of the food stream.

Korschelt ('89) observed in the egg of *Dytiscus marginalis* that the granules from the nurse cells enter the ovum and migrate in a broad stream toward the nucleus, which actually exhibits amoeboid movements, sending out pseudopodia-like processes toward the granules. These form changes, which were observed in both living and fixed material, are regarded as manifestations of an attractive force exerted between the nucleus and the granules. Somewhat similar processes were observed in the eggs of *Carabus*, *Bombus* and *Apis*.

I have not found such pronounced evidence of nuclear movement in either the living or fixed egg of *Leptinotarsa*. Fig. 11 represents conditions comparable to what Korschelt has described in *Dytiscus*, although the nucleus does not show any change in form. However, the facts are such as to indicate the presence of an attractive force of some sort, probably of a chemical nature, between the nucleus and food stream.

At the periphery of the mature egg and enclosing the yolk, is found a narrow layer of protoplasm continuous with the interdeutoplasmic cytoplasm. Since the yolk is elaborated from within out, being first formed in the center of the egg, it appears that the outer layer of cytoplasm together with the reticular part represent regions where the cytoplasm of the primordial germ cells has remained undifferentiated.

The evidence all goes to indicate that the substance of the food stream is identical with what has been called the "yolk nucleus" by a large number of writers, (Stuhlmann '86, Jordan '93, Balbiani '93, Calkins '95) in the eggs of spiders, myriapods, amphibia,

insects, and earthworms. In all these cases the action of differential stains indicates that the substance of the yolk nucleus is nearly related to chromatin, as I found to be the fact with the nutritive material in the egg of *Leptinotarsa*. The close proximity of the yolk nucleus to the germinal vesicle has led many workers to regard it as derived from the chromatin. Possibly when more complete data are at hand, it may be shown that in these forms, as in *Leptinotarsa*, the yolk nucleus is nothing more nor less than a nutritive stream that has its origin in cells outside of the ovum; that is, in the follicle or nurse cells, as the case may be.

It is rather interesting that the chromatin and the granules of the nutritive stream should show similar reactions toward the basic dyes. The granules are used in the formation of the yolk, and the product resulting from this transformation takes the acid stain. In the development of the embryo there is a reversal of this process. At fertilization the germ nuclei unite in the center of the egg, and the cleavage nuclei become scattered about in the yolk. These nuclei, which take the basic stain as intensely as the nutritive granules or the chromatin, then migrate in part to the peripheral layer where they form the blastoderm, and in part, remain in the yolk as the yolk nuclei. Through the activity of the latter, the yolk is converted into a form which can be assimilated by the growing embryo. Thus the agencies which are concerned with the conversion of the inert yolk into living protoplasm, the yolk nuclei, are somewhat similar in chemical make-up to the material from which the yolk is elaborated, the granules of the food stream. The entire process bears considerable resemblance to a reversible chemical reaction.

It is well known from the work of Loeb ('02) and Mathews ('07) that the eggs of the starfish, *Asterias Forbesii*, if allowed to mature in sea water in the presence of oxygen soon die, unless fertilized. Mathews has shown that the early death of the egg after maturation occurs only when free oxygen is present: from which it is concluded that death is brought about by the oxidation of the cytoplasm. Furthermore, this takes place much more rapidly after the contents of the germinal vesicle have been discharged into the

cytoplasm than before. If the nuclear wall remains intact, the egg does not become opaque even in the presence of oxygen. According to Mathews the sperm brings into the egg cytoplasm, which already contains an oxydase, two substances: a reducing agent, the centriole, which counteracts the action of the oxydase of the cell cytoplasm, and a very active nucleus which grows rapidly and forms more reducing substance, and possibly some oxydase. "By the entrance of the sperm there is set up that extraordinary series of opposite actions of oxydations and reductions which accounts for the sudden bursts of respiratory activity which probably underlies many of the most important syntheses and chemical transformations in protoplasm" (p. 107).

It has been shown by Fischer ('99) and others that the granules in the living starfish egg take the basic stain and are therefore electro-negative. It is also well known that a region of intense reduction will act as a negative electrode, from which it follows that granules staining with basic dyes are to be regarded as reducing substances.

The conditions in the growing egg of *L. signaticollis* are of course somewhat different from the material on which the above conclusions were based; but there are a number of very suggestive points of resemblance that should be considered.

In the first place, if one may judge at all from staining reactions, the food stream coming from the egg string consist of particles of a reduced substance which, through the activity of the oxydase in the cytoplasm, is converted into an inert oxidation product, the yolk. After fertilization every cleavage nucleus represents a region of intense reduction which reacts with the oxydized yolk converting it into living protoplasm.

This hypothesis rests on the assumption that the staining reaction of a substance is an indication of its chemical nature. A large number of investigators (Ehrlich '91, Mathews '98 and others) have demonstrated very clearly that staining with aniline dyes, depends upon a chemical union between the dye and the substance dyed. However, it often happens that in the process of fixation, the chemical reaction of the tissue is made opposite from what it was in the living condition, and this is especially true when the

salts of heavy metals are used. For this reason I selected picro-acetic acid as being least objectionable.

Any process of fixation produces chemical changes in protoplasm which make the fixed material quite different from the living. Whether or not a complete reversal in chemical reaction occurs depends upon the amount of nucleic acid present. If this is present in more than sufficient quantity to counter-balance the tendency of the killing fluid to make the protoplasm positive, the staining reaction would, of course, be the same as that in the living material. This is undoubtedly true of the chromosomes, and the evidence goes to show that it is probably true of these granules, although the proportion of nucleic acid in the latter is much less than in the former.

In any event the presence of a cyclical process with alternating phases of acidity and basicity is perfectly evident, since the same killing agent is used at all times, and its specific action may be assumed to be the same at all stages. The variable factor is the protoplasm; the changing proportions of the basiphil anion, nucleic acid, and the kation albumen radical, being responsible for the different staining reactions obtained.

Furthermore, these staining reactions are in keeping with what one would expect from the theoretical side of yolk formation. Yolk is regarded as an inert chemical substance, which implies a low degree of chemical activity. Its reaction to the stain shows it to be in an oxidized condition. The nutritive stream which is the product of living cells shows an opposite reaction, indicating chemical properties opposite to those of yolk. In the process of forming yolk the basic staining granules of the food stream disappear, and the acid staining yolk comes into existence as a product resulting from the interaction of nucleus, cytoplasm and food stream.

### *The Nurse Cells*

The nurse cells are descendants of the primordial germ cells whose reproductive function has been lost, and therefore they are to be regarded as aborted eggs. The first result of the differen-



tiation of the nurse cells has already been noted, and consists in the chromatin of the nuclei taking on a granular appearance, whereas in the young ovocytes the chromatin is in the form of a spireme. It has also been pointed out that the nurse cells undergo mitotic division for some time after the ovogonia have stopped dividing, but before the formation of the egg-string and the beginning of the growth period, mitotic divisions cease in those nurse cells situated next to the ovocytes. As these cells are the first to be called upon to supply nutrition to the egg, they are the first to take on the characteristic appearance of the nurse cells as found in the adult (Fig. 11). The first step in this process is brought about by the grouping of the cells into cyst-like structures, as shown in Fig. 15. These elongated cysts of polynucleated masses do not maintain their enormous size, but gradually break up into smaller pieces, even into parts containing a single nucleus, and therefore, morphologically equivalent to a single cell.

After the cysts have appeared, the epithelial cells are found in the spaces between the cysts, where their relation to the latter is the same as their relation to the primitive germ cells of early stages (Figs. 4, 6, etc.). This similarity of relationship suggests that the cells of each cyst are descended from a single mother cell, and examination of intermediate stages bears out this idea. In earlier stages as in Fig. 1 the definite relationship between germ and epithelial cells does not exist. However, as development continues each germ cell becomes surrounded by its own set of epithelial cells bringing about the condition shown in Fig. 37, which may be regarded as the first step in cyst formation. From this point, the germ cells, or rather the nurse cells as they now may be called, undergo amitotic divisions of the nuclei, and the resulting polynucleated mass remains enclosed by the epithelial cells. At first the lines of demarcation of the cysts are not sharp, but as the spaces which develop between them widen, the cysts become more rounded in outline and distinctly separated from one another (Fig. 16, which is a transverse section of an ovariole through the region of the nurse cells). This process begins among the lowermost nurse cells and gradually extends throughout the distal portion of the egg chamber. As the cysts appear at the



beginning of the growth period of the ovocytes, their formation is undoubtedly connected with the functional activity of the nurse cells.

Those of the primordial germ cells that develop into functional ova are not grouped into cysts, and epithelial cells are scattered among them very irregularly (Figs. 6, 7, 8, 9, etc.).

The formation of cysts does not have anything to do primarily with the differentiation of the primordial germ cells into nurse cells on the one hand and egg cells on the other, for in the testis where a similar process of cyst formation occurs, the cells thus inclosed develop into functional spermatozoa. Therefore the process of encystment can not be fundamentally antagonistic to the development of functional germ cells. In the case of the ovary, the differentiating factor has already operated before the cysts are formed.

In the course of cyst formation in the more distal part of the tube, one frequently sees mitosis and amitosis going on side by side (Fig. 13). It is interesting to note that here mitotic figures are never found inside of definitive cysts, where the multiplication of nuclei always takes place by the amitotic mitotic method. The products of mitotic cell division are single cells. The occurrence of irregularities, such as shown in Fig. 19, has been alluded to.

Aside from the absence of the mitotic division apparatus, the amitotic division figure is characterized by the fact that the nuclear membrane persists throughout the entire process (Figs. 20, 21, 22, 23). Further, the chromatin of the nucleus, which is in the form of rounded granules embedded in a reticular network, is separated from the nuclear wall by a clear space that disappears when the nucleus comes to rest after division (cf. Fig. 16).

The first authentic case of amitosis in the insect ovary seems to have been noted by Mayer (75), who observed the occurrence of doubly-nucleated cells in the follicular epithelium of *Pyrrhonoris apterus*. A little later, Brandt ('78) described in the nurse chamber of *Septura rubratisturea* "bisquit formige Kerne auf-treten die auf Theilungen hindeuten." Will ('85) described similar divisions of the nurse cells in *Nepa* and *Notonecta*, and used the facts to good advantage in developing his "Oöblasten" theory.

At about the same time, Carnoy ('85) noted amitotic divisions in the follicle cells of *Gryllotalpa* and a little later, Korschelt ('87) made similar observations in the case of *Hydrometra locustris*. As a result of studying the ovaries of *Nepa cinerea* and *Locustra viridissima*, Preusse ('95) described amitosis not only of the nucleus, but of the entire cell, and therefore claimed for the direct method of cell division an active part in the multiplication of cells. De Bruyne ('99), working in part with the same material, maintained that in no case does the division extend to the body of the cell, and that degeneration inevitably follows amitosis. Gross ('03) interpreted amitosis as he found it in eleven species of hemiptera in a slightly different manner. Its occurrence among the nurse cells is regarded as an indication of degeneration, but in the case of the epithelial cells of the egg follicles it has a deeper significance, for here it serves to enlarge the activity of the nucleus by increasing the area of contact between it and the cytoplasm.

The Ziegler-vom Rath theory (Ziegler '91, Ziegler and vom Rath '91, vom Rath '95) briefly stated is as follows: Amitosis appears in old and used-up tissue and consequently also in cells which have a transient significance. It occurs principally in cells which through very marked specialization take on the function of intense secretion or assimilation.

With the exception of Preusse, the unanimous opinion seems to be that amitosis as it occurs in the insect ovary is confined to division of the nucleus and is therefore not to be regarded as a method of cell multiplication. Thus Köhler ('07) says; "Die Kerntheilung der Nährzellen und Follikelzellen die amitotisch verlaufen, führen nie zu Zelltheilung. Diese Theilungen sind keine Vermehrungsteilungen sondern Differenzierungsteilungen. Sie bezwecken gar nicht eine Zellvermehrung und können deshalb in keinen Weise gegen die Möglichkeit eine propagativen Beweiskraft besitzen." This conclusion may be accepted as the general trend of opinion. Preusse's figures are far from convincing, and his conclusions regarding this point have been severely criticized.

In *Leptinotarsa* it has been shown that the large polynucleated masses eventually break up into single cells, and if this is to be looked upon as the last step of the amitotic process that started

in the nuclei, amitosis is here certainly concerned with cell multiplication. This protracted form of cell division is quite different from Preusse's descriptions, nor is it necessarily at variance with the observations of other authors; for unless a continuous series of stages are studied, it would never be suspected that the amitotic divisions which are at first confined to the nucleus, later extend to the cytoplasm.

The striking feature of the amitotic figure is the fact that the nuclear membrane does not disappear, but remains intact throughout the entire process of division. This fact together with the staining reactions serves to throw some light on the fundamental difference between mitosis and amitosis.

It has long been recognized that the dissolution of the nuclear membrane is in some way associated with a well defined alteration in the capacity of the egg for further development. From the observations of Delage ('01), it would appear that the essential feature of maturation is not so much the separation of the polar bodies as the removal of the barrier between the nuclear and cytoplasmic areas. The critical event which causes a change in the cytoplasm is the passage of nuclear constituents into it. The result may be a change in the osmotic pressure of the cytoplasm or in its rate of oxidation. Delage also observed that enucleated egg fragments of *Asterias* are incapable of fertilization before the germinal vesicle has broken down, but that very soon after the membrane shows signs of dissolution, merogonic cleavage becomes possible.

In his study of the karyokinesis of the *Crepidula* egg, Conklin ('02) arrived at conclusions which are important in this connection. "The nuclear membrane appears to permit the passage of materials inward, but not outward during the resting period, whereas the escape of nuclear material into the cell is brought about by the disappearance of the membrane during karyokinesis." He also determined cytologically "a very extensive exchange of material between the nucleus and cytoplasm. A large part of that most characteristic nuclear substance, the chromatin, passes into the cytoplasm during every cell cycle, while a relatively small part is reserved for the purpose of reproducing the daughter

nuclei." The passage of the nuclear material into the cytoplasm is regarded as a fundamentally important condition to the subsequent changes undergone by the latter.

Wilson and Mathews ('95) have shown that by far the greater part of the chromatin is set free in the cytoplasm in the first maturation division of the starfish egg, and F. R. Lillie ('06) states that in *Chætopterus* the greater part of the germinal vesicle consists of a "residual substance" which is set free in the cytoplasm of the first maturation division, and plays an important part in the future development.

If these phenomena are characteristic for mitosis in general, as they seem to be, the rupture of the nuclear membrane permits the escape of some substance into the cytoplasm that is essential to the changes which follow. Therefore, if the membrane remains intact during division, a difference in cell metabolism is certain to take place.

Lyon ('04) has shown that the production of carbon dioxide by the dividing egg follows a rhythm parallel with that of the nuclear division, and Loeb ('06) has connected these oxidations with the synthesis of nucleins from the compounds of cell metabolism—a process which likewise undergoes a rhythm parallel with that of the mitotic process.

Mathews ('07) suggests that the periodic dissolution of the nuclear membrane in mitotic cell division might have the significance of providing for the distribution of the oxydases (synthesized in the nucleus) through the cytoplasmic area which would naturally result in a periodic acceleration of oxydative processes in the cell.

R. S. Lillie ('08) points out that as certain enzymes exhibit the properties of nucleoproteins, it is reasonable to regard the so-called "oxychromatin" or "residual substance" as consisting, at least in part, of ferments concerned in the chemical processes—largely oxidative in nature as shown by the condition in the starfish egg—that determine the later characteristic changes in the cytoplasm. In this way the physiological data can be readily reconciled with cytological observations.

The failure of the nuclear membrane to dissolve in the course

of amitotic divisions in the nurse cells would result in the cytoplasm being deprived of the proper amount of oxidative ferments, and owing to the diminished rate of oxidation in the cytoplasm there would be an accumulation of unoxidized substances. The evidence derived from staining reactions justifies such a prediction.

At about the time of the fragmentation of the large polynucleated masses, a change occurs in the staining properties of the cell constituents toward aniline dyes. Up to this point the nucleus takes the basic dye while the cytoplasm takes the acid; but now a complete reversal is to be noted, the nuclei staining a deep green, while the cytoplasm, filled with the granules of the food stream, stains deeply with the red (Fig. 24).

The change is not an abrupt one, but begins gradually, shortly after the cessation of the amitotic divisions of the nuclei when the latter pass into a kind of resting state. Just before the change, one or more large basic-staining granules about the size of a nucleolus appear in the cytoplasm either closely applied to the nuclear membrane or at various distances from it (Figs. 14, 24, *gr.*) In younger stages these granules are found inside of the nucleus. As conditions between the intra- and extra-nuclear position of these bodies are not wanting, it seems clear that they arise in the nucleus. Whether or not these granules represent part of the chromatin contents of the nucleus that is being cast out into the cytoplasm as a result of degeneration or intense secretory activity, is a matter of speculation. It is certain that the chromosomes never appear subsequently. On reaching the cytoplasm the granule either breaks up into smaller particles or dissolves gradually without first disintegrating.

The smaller basic-staining granules of the food stream are found at the nodal points of a reticular network (Fig. 24, etc.). The latter is possibly an artifact, but the granules are probably present as such in the living egg, being comparable to the zymogen granules of the pancreas.

Thus, as far as the staining reactions are concerned, the nucleus and cytoplasm have exchanged places, and this peculiar inversion of the natural order of things seems to be the result or accompani-

ment of amitosis when continued for a number of cell generations. It has already been pointed out that the basic-staining property of the cytoplasm is probably due to incomplete oxidation of substances which consequently remain in a reduced condition. Inside of the nucleus on the other hand, the retention of the oxydases results in a high degree of oxidation; hence the capacity of this region to stain with the acid dye.

If these changes in chemical properties are due to the failure of the nuclear membrane to disintegrate periodically, we can readily see how amitosis in the nurse cells is related to the preparation of the basic-staining granules of the food stream. Furthermore this conclusion is in perfect keeping with the physiological observations of other workers which support in every way the interpretation put forth here.

We now come to a series of phenomena which represent a most peculiar form of cell activity. Shortly after the red nucleolar granules are extruded from the nucleus, one notices here and there a basic staining spot in the green nuclei of these cells (Figs. 27). Ordinarily, this does not occur in a singly-nucleated cell, but in one of the nuclei of a polynucleated mass (Fig. 25). This spot increases in size and forces the green area to the periphery (Fig. 28). Finally as a result of the continued expansion of the central part, the original nucleus and cytoplasm become reduced to mere shells (Fig. 31); but before it has reached its largest size, a green area appears in the center of the central red area as shown in Figs. 25, 26, 29, 30, 31. About this time, or even before (Fig. 28), the cell with its concentric layers usually becomes pinched off from the polynucleated mass (Figs. 26, 31). Fig. 32 shows a peculiar condition in which the central part (nucleus?) is dividing.

This remarkable series of changes begins in the distal region of the ovariole at about the time the first batch of eggs are half-grown. Since all of the nurse cells do not pass through this cycle of changes, the phenomena can not be regarded as degenerative processes, nor would they seem to be concerned with the elaboration of nutritive material, for the reason that so few cells are involved.

The fact that in the final stage as shown in Fig. 30, the central

area with its green nucleus bears some resemblance to an egg in early stages, led to the suggestion that this might be a part of the regular development of the egg. However, I soon found that most of the eggs do not undergo such transformations, but develop as has been described, from the germ cells at the base of the chamber.

Inasmuch as these peculiar configurations are of no significance as manifestations of the general degeneration associated with specialized function of the nurse cells, or in the production of the nutritive stuff for the eggs, or lastly, in the normal process of egg-building; I have reached the conclusion that the process is one of phylogenetic significance, in which certain of the nurse cells are passing through alternating conditions of oxidation and reduc-

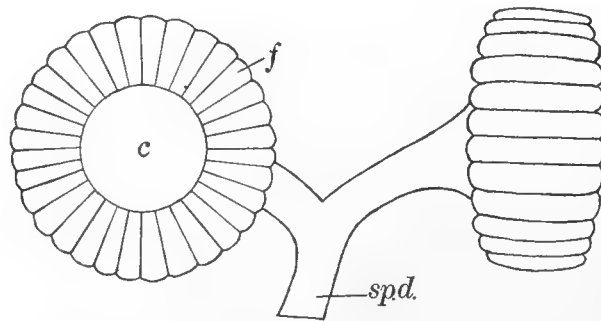


FIG. Y. Semi-diagrammatic drawing of the testis, one of the lobes being turned through an angle of ninety degrees. *c*, cap-like region opposite the sperm duct; *f*, follicle; *sp. d.*, common sperm duct of one side.

tion similar to those undergone by the egg (for it is to be remembered that these cells are really germ cells that have lost their reproductive function). There is no evidence that functional eggs result from this process. It can be readily shown that the eggs that are differentiated at the base of the chamber do not exhibit these changes. The possibility of two methods of egg formation might be considered, but there is no indication of dimorphism in the offspring, such as would be expected. On the other hand, the possibility of reproductive cells from either source producing identical offspring, meets the objection that there are more than enough germ cells differentiated at the base of the egg chamber to account for all of the eggs laid.

To my knowledge, the literature is without data bearing on these later transformations in the nurse cells. Korschelt ('86) described conditions in the young eggs of *Dytiscus* which resemble these changes to a certain extent, but his descriptions are too meager to admit of much comparison. I am inclined to think that most workers have regarded such appearances as the result of degeneration, and have, therefore, passed over them as of no particular interest.

## II. THE DEVELOPMENT OF THE TESTIS

The testes of the adult consist of a pair of bean-shaped lobes on either side of the body (Fig. Y). Two ducts, one from each lobe, unite to form a common duct (*sp. d.*) on one side, which in turn joins with its fellow from the other side to form the median ejaculatory duct. Internally each lobe is made up of radiating follicles, containing cysts of germ cells in various stages of maturation, while in the center is a lumen filled with mature spermatozoa during the breeding season. No suspensory ligament comparable to the end thread of the ovariole is present, the organ being supported by the fat bodies, tracheæ etc., which are packed about it.

In the early stages of development the organ bears but slight resemblance to the adult, each lobe being somewhat spindle-shaped and looking very much like a single ovariole at a corresponding stage (Fig. Z, A).<sup>1</sup> Histologically, as in the ovary, two kinds of cellular elements can be distinguished—germ cells and epithelial cells (Fig. 17, 18). Here too, there is the same tendency for several of the latter to be grouped around a single germ cell (Fig. 36). Fig. 17 shows the first stage in cyst formation in which, as in the ovary, the epithelial cells exhibit the same relationship to the completed cysts as they do to the single germ cells of earlier stages. The contents of each cyst are the descendants of a single mother-cell.

<sup>1</sup>Each lobe terminates in a cap of epithelial cells resembling the expanded base of the terminal thread of the ovariole of the adult female. A consideration of the significance of these cells together with a more complete description will be given in a future publication.



Sections of the late larval testis show that cyst formation has already occurred in the proximal part of the testis, *i. e.*, the part nearest the sperm duct, while in the more distal region they have not yet formed.

The spindle shape of each lobe persists during the greater part of the larval stage. The first change consists in an increase in diameter, while the length remains practically the same, which results in the production of a pear-shaped body (Fig. Z. B). During the pupal period the growth at right angles to the original axis of the lobe continues, but not equally in all directions, being inhibited at certain points, and producing a structure which in section resembles the hub and spokes of a wheel. The process continues in this manner until the radiating follicles characteristic of the adult are formed (Fig. Y).

At the point opposite from where the sperm duct leaves each lobe, a button-like cap exists (Fig. Y. c) in which the cells are very much younger than in any other part of the testis. This region represents the distal end of the embryonic organ, as can be seen from the method of development.

The epithelial investment covering the organ is at first very loose and does not extend in between the follicles, and when the testes are removed from the body at this time, the delicate epithelium falls away and the naked organs are obtained. In late pupal stages, the epithelial cells become more closely applied, and adhering between the follicles, soon produces the appearance characteristic of the adult, in which the follicles are separated from each other by a thick layer of these cells. Through increased number of cell divisions and the growth processes accompanying the maturation of the germ cells, the follicles become greatly enlarged and press tightly against the epithelial partition causing the latter to appear as a definite part of the follicle.

In connection with the early stages in the development of the ovary, the occurrence of amitotic divisions in the primordial germ cells was noted. At a corresponding stage the same phenomenon is to be seen in the testis. Three steps in the process are shown in Figs. 67, 68 and 69, the appearance of which closely resemble Figs. 34 and 35 from the ovary.

When about to divide, the nucleolus appears as an abnormally large basic-staining body surrounded by a perfectly clear area that serves to intensify the vividness of the dye. Fig. 68 shows the separation of the daughter halves during which process the clear area takes the shape of an hour-glass. The constriction then extends to the nucleus itself (Fig. 69), and eventually to the entire cell.

The amitotic period for any given cells is not of long duration, not more than perhaps several cell generations; although this could not be determined very precisely. It begins in the larval period at about the time of cyst formation, with the early development of which it is concerned. At any rate, in the earliest stages at which the cysts can be recognized, they are filled with cells undergoing amitosis. A little later these same cysts are filled with mitotic spindles, after which no evidence of direct division is to be found. It is thus seen that the early stages in the building of cysts in the testis are practically the same as those described for the formation of the analogous structures among the nurse cells of the ovary.

While the appearance of the amitotic figure here is entirely different from that found in the nurse cells of the ovary, there are several points of resemblance. In the first place, the nuclear membrane remains intact in both cases. Secondly, the chromatic part of the cell is surrounded by a clear region; extra-nuclear in the case of the nurse cells, and intra-nuclear in the case of the primordial germ cells of both ovary and testis. Thirdly, in both, the process is connected with cyst formation, except in the primordial germ cells of the ovary.

Perhaps the most striking differences are that in the nurse cells direct division continues indefinitely, resulting in certain chemical changes in nucleus and cytoplasm, and that it is not followed by mitosis; while in the germ cells it is, of relatively short duration and is followed by mitosis: the cells developing into functional germ cells. Evidently the process does not have the same significance the two cases.

The fact that the amitotic divisions occur in the germ cells of the ovary for only a very brief period makes it somewhat difficult

to detect the process. In the testis it is more readily discovered, for here, even in the adult, when the organ is packed full of spermatozoa, amitosis can be seen among the spermatogonia lying in the cap-shaped region opposite the point where the sperm duct leaves. Many of these cells have not yet been grouped into cysts, and all graduations between this condition, which is really characteristic of the larval stages, to well defined cysts, can be found.

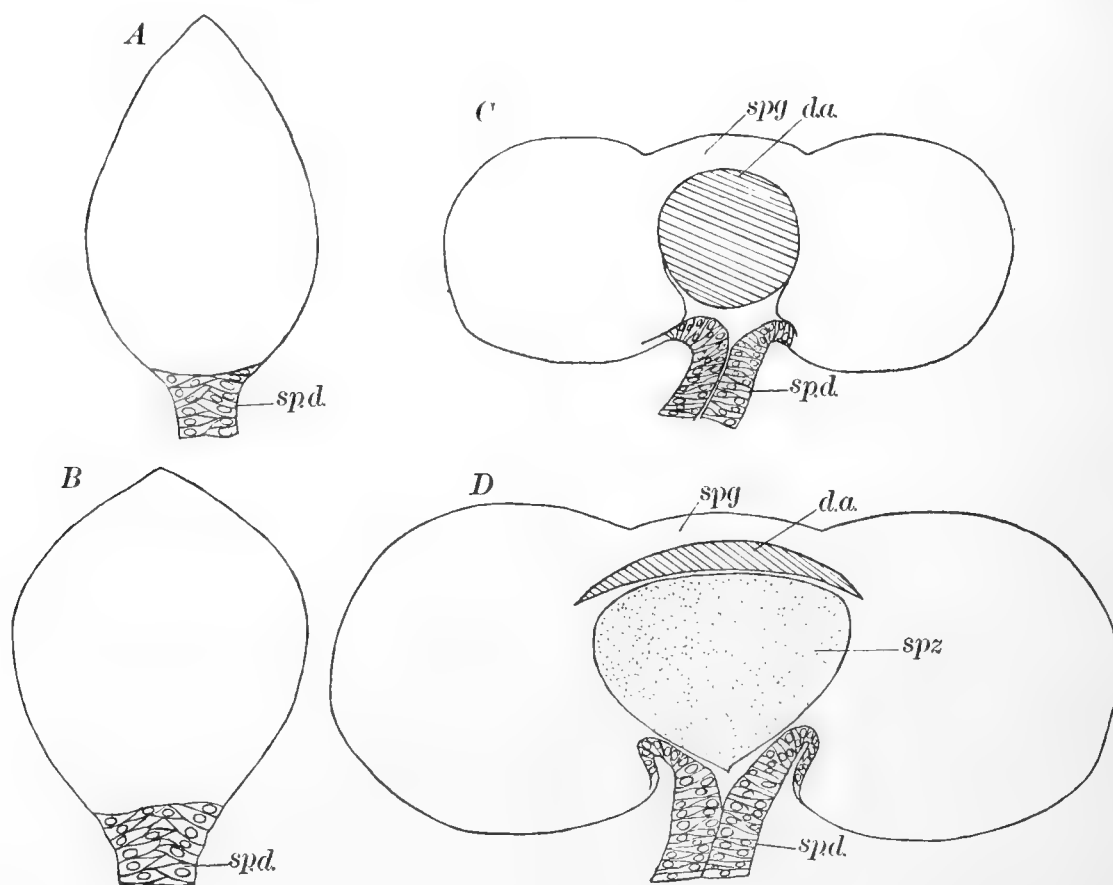


FIG. Z. Diagram of longitudinal sections showing four stages in the development of the testis. *A*, larva; *B*, larva two days older; *C*, pupa; *D*, adult. *d. a.* area of degeneration; *sp. d.* sperm duct; *spg.* spermatogonia, filling cap-shaped region opposite the mouth of the sperm duct; *spz.* spermatozoa.

In the examination of a large number of sections of the adult testis, I was surprised to find in all of them certain definite regions in which degeneration of cells occurs. Further study showed that the phenomenon is a regular event in the development of the organs. (I have yet to discover a testis in which it is not found.)

When adult stages such as represented in Fig. Z, D, are examined, the degenerated area (*d. a.*) is seen to lie directly beneath the button-shaped region (*spg.*) and to be encapsulated in a definite covering of epithelial cells, which sharply mark it off from the surrounding tissues.

The process begins in the larva and extends throughout the pupa and adult stages. The first step is an accumulation of epithelial cells in the center of the testis, reaching from the opening of the sperm duct back to the cap-shaped area (Fig. Z, C). The region becomes filled with irregular cell fragments that stain deeply with basic aniline dye or with iron-alum-haematoxylin. The cells remaining intact take on a shrivelled and wrinkled appearance and exhibit indications of amitosis, although the latter could not be made out very distinctly, owing to the shrunken condition of the tissue. As the walls of the sperm duct (*sp. d.*) grow into the testis, the degenerated area retreats, and finally becomes encysted by the surrounding epithelial cells.

Fig. Z, D, is from a young adult and shows the relation of the degenerated area to the surrounding regions. Above it at *spg.* is the cap-shaped part filled with spermatogonia, and below it is the general cavity of the testis packed full of spermatozoa (*spz*).

I have noted this curious condition not only in *L. signaticollis* which had been reared in breeding cages, but also in wild *L. decemlineata*, collected from potato vines growing in the open. Therefore the process can not be regarded as a pathological one produced by unnatural conditions accompanying confinement in a cage, but is an event that has a normal physiological significance, which will be considered presently.

Amitosis in the primary spermatogonia of certain amphibia has been described by La Vellette St. George ('85), Meves ('91), Benda ('93), and McGregor ('99), and the descendants of these cells are said to become functional spermatozoa. King ('07) states that in *Bufo lentiginosus* amitosis never occurs. An irregular outline is characteristic of the nuclei of the primary spermatogonia, but the constrictions never lead to actual division. Nussbaum ('01) describes "maulbeerformige Kerne," a condition which he maintains is merely a step in the process of mitotic cell

division and not an indication of amitosis or degeneration. This author observed nuclei whose sinuous outlines simulate stages of direct division in the egg of *Rhabditis nigrovenosa* and *Ascaris megacephala*, and in the spermatogonia of *Rana fusca*. These cells in all cases subsequently divide mitotically.

On the other hand, the work of Gerassimow ('92), Nathanosohn ('00) and Haecker ('00) demonstrate experimentally at least, that mitosis may follow amitosis and *vice versa* in both plants and animal cells. In these experiments it was found that under the influence of low temperature or narcotics, mitotic divisions cease, and amitosis ensues. When the cells are brought back to normal conditions mitotic divisions reappear.

Child ('07 c) has reported the occurrence of amitosis in the representatives of six different animal phyla, in practically all kinds of tissue including the reproductive cells. In general, his observations indicate that amitosis is more frequent than mitosis in connection with a rapid cell multiplication which accompanies normal and regulatory growth. Child speaks of mitosis as a cyclic process in that the nucleus, starting from the resting stage, undergoes during every mitotic division a series of changes and finally returns to a resting stage similar to the starting point. In amitosis there is no such cyclic movement. "Nothing in the visible phenomena indicates the occurrence of reversal in direction of the processes involved." In other words the nuclei of these cells are not in conditions of equilibrium with the cytoplasm.

The most interesting of his observations (Child '07 a, '07 b) deal with the development of the ovaries and testes of *Moniezia*, in which the early divisions of the germ cells are amitotic. Then comes a growth period at the beginning of which a spireme is formed, followed by the maturation divisions, which take place mitotically, as do those of the early cleavage. The remaining divisions are amitotic.

Patterson ('08), from observations made on the blastoderm of the pigeon's egg, came to the following conclusions. 1. Mitosis may follow amitosis and *vice versa*. 2. Amitosis is the result of a physiological stimulus which creates a stimulus to growth. 3. Amitosis exists in regions of rapid growth. Maximow ('08)

described amitotic cell division in the stellate cells of the body mesenchyme. Like Patterson and Child, he believes it occurs in regions of rapid cell proliferation, and attributes to it a normal physiological significance of some kind.

The occurrence of amitosis in so many different forms and such a variety of circumstances makes it imperative that this type of cell division be recognized as a factor in normal developmental processes. In view of so many accounts, only a few of which are mentioned above, the fact of its existence must be generally recognized, but the question of its significance and relation to mitosis remains without a satisfactory answer.

In the species under consideration, I have described two distinct types of amitosis. In the case of the nurse cells, the process is evidently concerned with the differentiation of these cells for a highly specialized function. Mitosis never occurs afterward. The existence of the direct form of cell division under these conditions has been accepted without much question since it was regarded as a species of degeneration, and further, was in no wise antagonistic to the hypothesis of the individuality and continuity of the chromosomes. In other cases where the claim has been made that mitosis may follow amitosis, so long as only somatic tissue was involved, no very serious objections have been made. However, when we come to instances of amitosis among the germ cells that later develop into functional reproductive cells, the supporters of the chromosome hypothesis have found it very difficult to accept the results of such observations.

Bearing in mind the theoretical interest centering about this point, I have been very careful to examine the ground thoroughly before stating definitely the occurrence of amitosis in the germ cells of *Leptinotarsa*. I have already pointed out that it is merely transient and inconspicuous in the ovogonia. In the spermatogonia it is more prominent, persists longer and is involved in the formation of the cysts.

The experiments of Gerassimow ('92), Nathansohn ('00), and Haecker ('00), have suggested to me that disturbances in the nutrition may be responsible for the amitotic period; the effect of narcotics and low temperature being similar to what one might

expect to result from a reduced oxygen supply. A very rapid increase in cell multiplication would cause a temporary diminution in the oxygen supply for each individual cell. The stimulus to increased cell division is a distinct factor that is bound up in the process of building cysts of the testis. As soon as the sudden increase in number of cell has been compensated by a corresponding increase in oxygen supply, the conditons required for mitosis are restored.

Child's observations upon the cestode *Moniezia* offer further suggestions along this line. This form is not only relatively low in the animal scale, but one which, in addition has undergone degeneration: a combination of circumstances which would lead one to expect primitive methods in cell division as well as in general metabolic processes. Here amitosis appears to be the regular method of cell division, while mitosis is comparatively rare, occurring only during the maturation period and early cleavage stages.

What causes the change from mitosis to amitosis? It has appeared to me that here likewise a gradual diminution in nutrition is responsible. It might be assumed that the object of the long rest stage or growth period in the development of the ovum is to elaborate food and formative materials for maturation, fertilization and embryonic development. We might further assume that the same process provides for a certain number of mitotic divisions extending through the early cleavage. The direct method of cell division sets in because of a deficiency in the amount of nutritive material (oxygen?) necessary for continued mitotic divisions. This is in keeping with the fact that amitosis occurs usually under abnormal metabolic conditions which are unfavorable to normal metabolic processes. Amitosis might be regarded as a simpler form of cell division, not so much because it takes place in the absence of spindle and chromosomes, as for the reason that it can occur under circumstances that make mitosis impossible.

In accordance with this idea one notices that amitosis has been observed most frequently under conditions of rapid growth, where if this explanation is applied, the cell makes use of the direct method of division rather than follow the slower and more complex mitotic method largely because of limitations in the way of

nutrition. The same principle can be applied in the case of the nurse cells. When the latter are being differentiated for their specialized function, very rapid and prolonged amitotic divisions occur. In degenerating cells amitosis is to be expected, because in many cases at least, the cells or tissues do not receive their normal oxygen supply.

Amitosis in the higher forms as a survival of a primitive process of direct division from the protozoa was suggested long ago by Strassburger ('82) and Waldeyer ('88), but the view has never met with much favor. This has been largely because of the popular and growing tendency since that time to associate amitosis with processes of degeneration and specialization, reserving for mitosis exclusively the role of cell multiplication in normal processes. However, there is much to support the older view, and the evolution of the mitotic method of cell division is, in a way, the expression of the evolution of a higher type of cell metabolism than that found in the lower forms. The *raison d' être* of the mitotic figure must rest upon a physiological basis, and the complexity of the mitotic cycle appears to be associated with a corresponding complexity of metabolism of a higher order than that found in cells that divide amitotically. In this sense amitosis in the germ cells of the testis and ovary of *Leptinotarsa* can be regarded as a temporary reversion to an ancestral method of division but the direct cause I believe, lies in some disturbance in the cell metabolism which occurs periodically in the ontogeny.

The appearance of the amitosis division figure is by no means the same in all cases, and this is of importance from the phyletic stand point. In nearly every instance, the process indicates a division of the nucleus into two approximately equal parts, but among the germ cells the mechanism is more carefully adjusted for this purpose (cf. figs. 21, 22, 23, 34, 35, 68, 69, etc.) Thus in the ovogonia and spermatogonia division of the nucleus is preceded by a very exact division of a large chromatin nucleolus, and as the halves separate surrounded by the clear area, the appearance reminds one very much of the division of a chromosome on a spindle. In fact, the process suggests a very primitive method of karyokinesis. Meves ('91) and Benda ('93), in *Salamandra*



have described a mechanism which may have the same significance. Here the direct division is said to be brought about by the constricting power of a ring-shaped centrosome.

The fact that in both the germ and nurse cells, the daughter nuclei are of approximately equal size, indicates the presence of a division mechanism of great precision and accuracy. The power or force involved in karyokinesis therefore, may not lie in the visible structures—centrosome, spindle, chromosomes, etc.—but in some invisible factor that is a property of the living protoplasm.

Mitosis and amitosis are often regarded as representing antithetical conditions, but, as a matter of fact, these two methods of cell division really stand for the extremes of a graded series. A simple type of amitosis is that shown in the nurse cells, where there is no evidence of any division apparatus. The nucleus undergoes a simple constriction and divides. In the case of the germ cells, the presence of the nucleolus, its exact division and the occurrence of the surrounding pale area, point to what might be called a higher type of amitosis. Another advance is seen in the germ cells of amphibia, where the constriction centrosome divides the nucleus (Meves and Benda). In many of the so-called mitotic division figures of the lower forms, the spindle is far from conspicuous and in many cases is represented by only a few strands of achromatic substance. In the division figure of the macronucleus of *Spirochona* and *Actinosphaerium* as figured by R. Hertwig ('79, '84), the spindle and equatorial plate are formed inside of the nuclear membrane. In *Spirochona* a hemispherical "end plate" or "pole plate" is situated at either pole of the spindle. Hertwig claims that these arise by a division of a large nucleolus whose behavior reminds one of the large chromatin nucleoli of the germ cells of *Leptinotarsa*. Keuten ('95) has demonstrated the origin of similar pole plates from nucleoli in *Euglena* and Schaudinn ('95) in *Amoeba*. *Euglena* presents a very primitive type of mitosis, spindle fibers being scarcely recognizable.

Among the metazoa, examples are to be found in which the mitotic figure is equally primitive, as in certain aphids, where according to Tannreuther ('07) no chromatic spindle occurs in the maturation division of the egg. Definite chromosomes of

constant size and number are found, but no astral radiations appear.

Furthermore, it is to be noted that among the protozoa and unicellular plants where mitosis may be said to exist in its most primitive form, the nuclear membrane remains intact and does not disappear at any stage of the division. This condition is characteristic of amitosis while on the other hand rupture of the nuclear membrane at some stage is an accompaniment of mitotic division. Again, in the lower forms the arrangement of the chromatin granules to form chromosomes appear to be of secondary importance as compared with the higher forms and the essential feature in nuclear division appears to be the fission of the individual granules.

These facts all point to the origin of mitosis from a primitive amitotic method of divisions, and therefore give considerable ground for a phylogenetic interpretation of amitosis.

Owing to the usual association of amitosis with degeneration, I at first thought that the degenerated area in the testis would furnish an explanation of the direct division in the early spermatogonia. I soon found, however, that only epithelial cells participate in the degeneration, and that so far as I was able to determine, the spermatogonia undergoing amitosis later develop into functional germ cells.

What then is the significance of the degeneration? The phenomenon recalls certain peculiarities in the development of the ovary. In the first place, it might be compared with the changes following the accumulation of epithelial cells at the base of the terminal chamber, which results in the effacement of the sharp line of demarcation between the latter and the cells of the tube stalk; but the appearance of the cells in the two cases is entirely different. In the ovary, the nuclei of the cells involved undergo a kind of liquefaction, whereas in the testis the cells disintegrate into irregular fragments.

Physiologically the process may be analogous to the change which converts certain of the germ cells of the female into nurse cells. The degenerated area of early stages is actually much larger than later on when it is represented by the part enclosed in the cyst,

which contains only cell fragments. These fragments are the remains of the more solid parts of the cells, the liquid constituents having been separated and secreted into the general cavity of the testis where they probably serve as a nutrient medium for the spermatozoa. This is suggested by the fact that the cavity of the testis is at first occupied by the degenerated area, and as the latter retreats toward the distal end of the testis, the former gradually comes into existence as the continuation of the lumen of the sperm duct. As a result then of the process of contraction, the fluid contents of the degenerated area would be expressed into the central cavity of the testis. The degenerated area might of course be regarded as a general source of nutrition for the germ cells in all stages of development, instead of merely the mature spermatozoa.

The degeneration then may be of significance in connection with nutritive processes; but it is to be remembered that the cells involved are not homologous with the nurse cells of the ovary, since the latter are derived from the primordial germ cells. This is not necessarily an objection, but is only in accordance with what might be expected. The spermatozoa are produced in far greater numbers than the ova. In order for this to happen it is essential that all or nearly all of the germ cells of the male develop into functional spermatozoa, none, if any being reserved for the function of supplying nutrition. That would throw this function on the only other element of the testis, namely, the epithelial cells. In the ovary where the course of evolution has taken a different trend, a large number of relatively small and simple ova has come to be not so essential as a small number of greatly enlarged and highly complex germ cells, as a result of which a considerable number of non-functional germ cells become the nurse cells of the ovary.

### III. THE CHROMOSOMES

The chromosomes of *L. signaticollis* are not of a size and number to make the material the best for a satisfactory study of all stages of the maturation process, but they present a number of features

that can be readily examined, and that are of interest and importance in view of recent developments in this field.

A polar view of a first spermatocyte spindle at metaphase shows the presence of sixteen chromosomes of various sizes, whose bivalent condition is indicated by a deep groove (Fig. 56). A longitudinal section of the spindle at anaphase shows at one pole a thick V-shaped body that stains red in safranin-lichtgrün, preparations exactly like the chromosomes (Fig. 54 *x*). Of the chromosomes, at the middle of the spindle, there is always one very much larger than the others, consisting of two L-shaped parts placed end to end. In polar views this chromosome can be readily identified by its large size. From the telephase shown in Fig. 57, it is evident that an unequal distribution of chromatin has taken place, one of the daughter cells receiving the odd body in addition to the sixteen chromosomes. This odd body is never found at metaphase with other chromosomes. In Fig. 55 it is shown at *x*, but in order to see it the focus had to be changed. This figure shows its usual position relative to the other chromosomes, which as a rule it precedes to the pole of the spindle, although it occasionally lags behind them.

When we come to work out the history of this odd body, it is found to have its origin in the resting stage following the last spermatogonial division, as a deeply basic-staining nucleolus of a bi-partite form (Figs. 48 and 49), which persists throughout the resting period when the ordinary chromosomes are represented by an irregular reticulum.

Spermatogonial equatorial plates show the presence of two homologous series of chromosomes, the number of which can not be determined satisfactorily at this stage by counting. Following the last spermatogonial division, the cells have the appearance represented in Fig. 45, in which the chromatin is arranged in the form of a split spireme that is closely contracted at one side of the nucleus. Generally at this time, a structure bearing an unmistakable resemblance to the odd body of the first maturation spindle can be seen (as in Fig. 39 although this figure represents a young ovocyte). From this we go to the stage shown in Fig. 46, where the spireme is becoming disentangled. The double nature

of the spireme can now be made out very distinctly, and the bilobed body is seen at *x*. In Fig. 47 is shown a slightly older stage in which the chromatin is collected in knot-like aggregations that manifest a diminishing tendency to unite with the basic dye, while the odd body or chromatin nucleolus, as it now may be called, retains its basic-staining capacity and becomes more prominent by contrast.

In Fig. 48, a reticular structure can be made out, at the nodal points of which can be seen small collections of chromatic material. Fig. 49 marks the end of the resting stage and shows the reticulum very jagged and irregular. It will be noticed that the nucleolus is attached to the ends of two parallel threads, and this is the first step in the reconstruction of the spireme of the prophase of the first maturation division. In Figs. 50 and 51, this spireme is seen to be composed of two homologous parts, each containing the reduced number of segments. From its position in close contact with the nuclear membrane, the nucleolus of the resting stage (*x*) can be readily distinguished from the segments of the spireme which it greatly resembles, (Fig. 50.) In Fig. 51, its identity is not so certain. Figs. 52 and 53 are prophases in which the spireme is breaking up into the reduced number of bivalent chromosomes plus the nucleolus, resulting in the production of seventeen bodies, each showing a transverse constriction. All except the nucleolus soon show the presence of another constriction at right angles to the first, forming the tetrads of Fig. 53. Here the nucleolus or odd body is shown in its characteristic position even before the spireme has completely segmented. It shows nothing of the quadri-partite condition of the other chromosomes that is indicative of the two subsequent maturation divisions.

The succeeding stages (Figs. 54, 55, and 56) have already been described. In Figs. 59 and 60 are shown the metaphases of the second division. In the former, seventeen chromosomes, including the odd body, (*x*) are represented, and in the latter, sixteen. No anaphases sufficiently clear for counting or drawing were found, but it is evident from Fig. 58 which is a characteristic second division telophase, that no uneven distribution of the chromosomes takes place, so that it is inferred that the bi-partite body divides, either transversely or longitudinally.

There is nothing of especial interest in the remaining details of the spermatogenesis except one stage in the development of the spermatozoan, where the head is seen to be made up of sharply defined basic-staining spheres whose number I estimated to be the same as the reduced number of chromosomes (Fig. 61). A little later this configuration disappears, and the head of the mature spermatozoan shows a smooth solid basic staining mass of the usual pointed form characteristic of *Coleoptera*.

As has already been mentioned the tangled condition of the chromosomes in the spermatogonial plates makes a direct determination of the unreduced number almost impossible. However, it is clear from the maturation spindles that the number must be thirty three or thirty four, depending upon whether the odd body is to be considered equivalent to one or two chromosomes.

At this point, I should like to call attention to certain appearances in the cytoplasm at the beginning of the growth period, that are suggestive of a process comparable to yolk formation in the ovocyte. As seen in sections, this consists of a crescentic acid-staining area traversed by a coarse irregular fibrous network (Fig. 49) that grows until it nearly fills the cytoplasm (Figs. 50, and 51). When the nuclear membrane disappears for the first maturation division, this material becomes diffused throughout the entire cell and loses its distinctness. It is interesting that its staining reaction is similar to that of the yolk of the egg.

The appearance of the resting stage recalls at once the condition first described by Gross ('04) in *Syromastes marginatus*, in which he observed a bi-partite nucleolus that arises by the synapsis of two spermatogonial chromosomes. This constitutes the accessory chromosome which from its mode of origin is bivalent. It divides longitudinally in the first division, but fails to divide in the second, passing bodily to one pole in advance of all other chromosomes. The result is that all of the spermatid nuclei receive ten chromosomes and half of them, in addition, the accessory. He further supposed only those spermatozoa to be functional that contained the accessory, the others being regarded as comparable to polar bodies. From the numerical relations he believed the somatic

number to be twenty-two in both sexes. The fertilization formula is:

$$\text{Egg } 11 + \text{sperm } 11 (10 + \text{accessory}) = \text{oö sperm } 22 (\sigma^7 \text{ or } \sigma^+).$$

Gross complicated his conclusions by a very fanciful interpretation of the relation between the accessory and the microchromosomes, which I shall not enter into here.

Wilson ('09a) confirmed Gross's observation that in *Syromastes*, the spermatogonial number of chromosomes is twenty-two, and that in the second maturation spindle there is a bivalent heterotropic chromosome. He also pointed out that the bi-partite nucleolus of the resting stage is composed of slightly unequal parts. Since the accessory arises from two chromosomes, Wilson considers it equivalent to two in the maturation divisions. Therefore the number of chromosomes characteristic of the two classes of spermatozoa are ten and twelve respectively, both classes being assumed to be functional. In a later paper Wilson ('09c), the ovogonial number was found to be twenty-four. The fertilization formula is given as follows:

$$\text{Egg } 12 + \text{sperm } 10 = \text{oö sperm } 22 (\sigma^7).$$

$$\text{Egg } 12 + \text{sperm } 12 = \text{oö sperm } 24 (\sigma^+).$$

The fertilized egg then, contains two bivalent accessory chromosomes, which judging from the facts of the spermatogenesis one would expect to appear in the resting stage of the ovocyte as a quadri-partite or quadrivalent body. To determine this point in *L. signaticollis*, I examined series of sections of young ovocytes, and found shortly after the last ovogonial division, a stage identical with synizesis in the male in which there appears a *bi-partite* basic staining nucleolus of approximately the same size and equal in all respects to the body noted in the spermatocyte at a corresponding stage. This is represented in Fig. 39. In Fig. 40, the spireme is unwound and is a stage that is comparable to Fig. 47 in the spermatocyte. Figs. 41 and 42 are slightly older. In the latter the nucleolus is seen at the end of the widely separated parts of



a thin double spireme, which condition persists for quite a while. Fig. 43 is a drawing of a nucleus considerably older, in which the chromatin represented by the beaded strands is beginning to lose its affinity for the stain. Thus Figs. 39 to 43 inclusive in the female, present an interesting parallel to figs. 45 to 48 inclusive in the male, and show that the stages marking the beginning of the growth period are exactly the same in both sexes.

The important point is the presence of a bi-partite nucleolus in the resting stage of the ovocyte that bears a very striking resemblance to the body found at a corresponding stage in the spermatocyte.

Stevens ('06) found in *L. decemlineata* (*Doryphora decemlineata*), a closely related form, that the spermatogonial number is thirty-six. The nucleolus of the resting stage is described as an unequal pair, the members of which separate in the first division and divide equationally in the second. The great similarity between the telophases of the first division as represented by Stevens in Figs. 175 and 176 of her paper and the corresponding stage in *signaticollis* led me to examine the ovaries and testes of *decemlineata*. I found the nucleolus of the primary spermatocytes to accord with Stevens' description as far as the resting stage is concerned, but that its unequal components separate in the first division does not seem to be the case, and in this regard I cannot agree with her observation. Figs. 62, 63, and 64 show various stages in the first division, in which the behaviour of the accessory body is exactly the same as in *signaticollis*.

Stevens claims that the V-shaped body seen in the first spermatocyte spindles is the larger component of the unequal pair and that owing to its smaller size the lesser component is often concealed by the "V," "when the group has the appearance of an Orthopteran accessory".

In *signaticollis* there can not be the slightest doubt as to the accessory not dividing in the first division, and since the two species are so closely related (cf. Tower '06), some similarity in the behavior of this body is to be expected. That Stevens should have placed a different interpretation on this point is not strange in view of the cytological difficulties in the way of a satisfactory



study, for which purpose the material is much less favorable than *signaticollis*. With the conditions in the latter relatively clear to guide me, I am convinced that the odd body does not divide in the first division in *decemlineata*. Telophases like those shown in Figs. 62 and 64 would seem to settle this point very definitely. Furthermore, the accessory body even in the late telophases does not mingle with the ordinary chromosomes but occupies a position at one side. If a separation of the components occurred, one would expect both parts to behave similarly in this regard and remain apart from the other chromosomes at their respective poles, but such is not the case. At only one pole is an eccentric body to be found and this bears an unmistakable resemblance to the nucleolus of the resting stage (Figs. 64, 66).

If, with Stevens, we regard the unequal pair as made up of two somatic chromosomes, it follows that the equal pair of *signaticollis* is likewise equivalent to two chromosomes. In this event the spermatogonial number is thirty-four ( $2 \times 16 + 2$ ).

The evidence from *signaticollis* would perhaps favor the idea that the bi-partite body represents a single chromosome, since its appearance and behavior in the maturation divisions recalls the unpaired accessory of the *Orthoptera* and certain *Hemiptera*. On the other hand, the fact that in *decemlineata* the homologous components are of unequal size practically compels one to regard the accessory body as two chromosomes in both cases. The chromatin nucleolus of the ovocyte of *decemlineata* is composed of two parts of unequal size (Fig. 65).

It has already been pointed out that the odd body in all probability divides in the second spermatocytic division, but whether transversely or longitudinally can not be readily determined. At first I was inclined to believe that the division took place so as to separate entire chromosomes but this view leads to serious difficulties, since it would follow that three classes of spermatozoa would be produced. In *signaticollis* one-half of the total number would contain sixteen chromosomes; one-fourth of the total number, sixteen plus one component of the odd body; the other fourth, sixteen plus the other component of the odd body. The same kind of difficulty would be met with in *decemlineata*.

The other alternative, namely, that the odd body divides longitudinally in the second division is more in accord with well established observations in other forms, and furthermore meets with no objections in the appearance of the various division stages. If we accept this interpretation, the case is exactly similar to *Syromastes* except that the order of division is reversed, the bi-partite dividing in the second division instead of the first, thus giving two classes of spermatozoa, one with sixteen and one with eighteen chromosomes. An identical instance would be seen in *Phylloxera* (Morgan '09), where the odd body is equivalent to two chromosomes which divide only in the second division, and then longitudinally.

On this basis the somatic tissues of the female would contain two more chromosomes than those of the male, and the fertilization formula for *signaticollis* would be:

$$\text{Egg } 18 + \text{sperm } 16 = \text{oösperm } 34 \text{ (♂)}.$$

$$\text{Egg } 18 + \text{sperm } 18 = \text{oösperm } 36 \text{ (♀)}.$$

However, the tangled condition of the chromosomes in dividing somatic cells, prevented verification of these points by direct observations.

My observations of the maturation spindles of the egg are very incomplete, and I am unable to say whether or not there is an unequal distribution of chromosomes such as occurs in the first spermatocytic division. It is possible that while the bi-partite nucleolus appears in the ovocyte, at the same time as in the spermatocyte, its subsequent behavior in the maturation divisions is entirely different.

Is the nucleolus of the ovocyte the homologue of the nucleolus of the spermatocyte? The transformations accompanying the long growth period of the ovocyte make it a very difficult matter to follow the history of this body through to the maturation spindles. The last stage at which it can be satisfactorily made out is shown in Fig. 43, which represents a section of the nucleus of an ovocyte. From this there occurs a gradual transition to the condition seen in Fig. 44, where in place of one, five rounded basic

staining nucleoli are present. The chromatin has passed from the condition of beaded strands into a formless mass that takes the acid stain. Finally an acid staining ground substance is produced in which a number of basic staining nucleoli are embedded. What relation these nucleoli bear to the nucleolus of the resting stage I am at present unable to say.

There are a number of facts that argue in favor of the nucleolus of the resting stage being homologous in the two sexes. In the first place in pre-reduction stages in both cases, a nucleolus is present in the vegetative nucleus between mitotic divisions, but it is entirely different in appearance from the one characteristic of the growth period. It is often jagged in outline, and may be single or multi-partite, while the bi-partite condition of the nucleolus is strictly a feature of the resting stage.

Secondly, the bi-partite nucleolus appears in both sexes at the time of synizesis (Figs. 39 to 46). Wilson ('06, p. 22-23), speaking of *Anasa* and other *Hemiptera* states: "In the female no trace of a chromosome nucleolus can be found in the contraction stage of the synaptic period . . . . I can, therefore, only state that no chromosome nucleolus is present in the contraction period synapsis or in the early growth period, and even though it be present in later stages, which I think very doubtful, a wide difference between the sexes would still exist in respect to the earlier period." Evidently the conditions in *Anasa* and *Leptinotarsa* are not the same, for the presence of the nucleolus in the latter can be demonstrated without any difficulty.

Thirdly, the behavior of this body in relation to the other chromosomes in the early part of the growth period is the same in both sexes (Figs. 39, 40, 41, 42 46, 47, and 48).

The evidence all tends to show that this body is in some way bound up with the processes connected with the early part of the growth period. It persists in the ovocyte as long as the development of the latter is parallel with that of the spermatocyte, but as soon as the ovocyte begins to show signs of the highly specialized metabolism involved in its enormous growth, the nucleolus can not be distinguished from a number of other bodies that make their appearance at this time.

A knowledge of whether this bi-partite nucleolus represents one or two chromosomes may be of no great importance in itself, but it has a very important bearing in determining the relation of the nucleolus to the segments of the spireme in synapsis. The manner in which synapsis takes place in insects is scarcely at all understood. The critical stages are just the ones that are omitted in descriptions or passed over so hurriedly that the reader is usually left to follow his own choice in the matter.

Wilson ('09*a*) in a admirable series of photographs, has produced evidence against the idea supported by Montgomery ('00), Sutton ('02), Stevens ('03) and Dublin ('05) and others, that synapsis occurs in the closing anaphases of the last spermatogonial division. He has shown that in *Pyrrochoris* the number of chromosomes in the postphases following the last spermatogonial division is not the reduced number, but approximately the somatic number. Synizesis does not follow immediately but is separated by a long resting period.

The extreme synizesis stage in the male of *signaticollis* is shown in Fig. 45, where the chromatin is in the form of a tightly wrapped split spireme. The double nature of the spireme is seen to better advantage in Fig. 46, where it is unfolding. As the number of its segments is greater than the reduced number of chromosomes, synapsis in the sense of an actual fusion has not yet taken place.

In the ovocyte, following the last ovogonial division, practically the same condition occurs. It happens that in the particular instance shown in Fig. 39 the two halves of the spireme are separated; which indicates very clearly the double form of this structure. Similar examples are not wanting in the spermatocyte. As one studies the succeeding stages shown in Figs. 41, 42 and 43, it is clear that no reduction has yet occurred.

Actual conjugation of chromosomes does not take place in the last stages of the spermatogonial or ovogonial divisions, although the chromosomes at this time do seem to pair into two homologous series which in synapsis have the appearance of a split spireme.

It is rather questionable whether synizesis indicates an actual contraction of the chromatin; at least in the material under consideration. In Fig. 46 it seems to mark the unfolding or dis-

entangling of the chromatin thread which has remained practically unchanged from the telophases of the last pre-maturation division, while the nuclear cavity has been enlarged. Wilson ('09a) speaks of the nuclear reticulum in *Pyrrhochoris* as undergoing contraction, yet the actual contraction of the chromatic material is rather insignificant (as shown by his figures, Photos. 35-42 inclusive) compared with the expansion of the nuclear membrane. Likewise in the case at hand the chromatin has changed but slightly in bulk since the telophases of the pre-maturation divisions, and the apparent contraction seems to be due principally to an enlargement or vacuolization of the nuclear cavity.

Fig. 51 shows the characteristic appearance of the spireme of the prophase of the first division, when the thickened thread is composed of the reduced number of segments, each of which is double. The nucleolus of the resting stage forms one end of the series (Fig. 50), and its relation to the spireme is such as to suggest that each segment of the latter is homologous with it. That is to say, if the nucleolus represents two fused or closely united chromosomes, then each segment of the spireme can be regarded as a double chromosome. In other words, synapsis has taken place by side to side union of the chromosomes. On the other hand, if we regard the nucleolus as a single chromosome, then end to end union of the chromosomes has occurred; and in this case the groove separating the two halves of the spireme represents the line of a longitudinal division; whereas in the other it represents the line along which the chromosomes had come together in synapsis.

An examination of Figs. 46, 47, 40 and especially 42, shows that during synizesis or at least very shortly after, the split spireme bears a similar relation to the nucleolus. To each lobe of the latter is attached one of a double series of segments, the number of which cannot be counted, but which is more than the unreduced number. In fact, there is approximately twice this number, which means that the chromosomes had united to form a spireme the demarcation of which into segments foretells the two maturation divisions.

Throughout the period preceding the first maturation division,

the behavior of the two halves of the spireme is such as to indicate two more or less independent parts, as in Figs. 39 and 42, where the halves are widely separated, and Fig. 50, where the separation is partial. These facts, together with the relation of the segments of the spireme to the presumably bivalent nucleolus, point to a separate origin of the two components of the spireme, as opposed to their resulting from a longitudinal splitting of a single series of units united end to end.

On this basis the synaptic process would take place somewhat as follows: In the post-telophase stages of the last pre-maturation division, the chromosomes arrange themselves side by side in homologous pairs to form a long thin double spireme behind the accessory body, which arises from two chromosomes that, instead of elongating as the rest of them do, contract to form the nucleolus of the resting stage. The line of separation between the two homologous series indicates one of the maturation divisions. The other division (except in the case of the nucleolus which only divides once) is indicated by a transverse groove in each chromosome which produces the large number of segments seen in synizesis and stages immediately after (Figs. 39 to 47 inclusive). With the exception of the nucleolus, all trace of chromosome structure is lost during the resting stage. The first indication of a reconstructing process is shown in Fig. 49 by the appearance of two parallel strands upon which the chromosomes take their places as shown in Figs. 50 and 51.

The occurrence of side to side union of the chromosomes or parasynapsis (Wilson '09a), has a bearing on certain theoretical aspects of maturation. It can be readily shown that the two divisions take place at right angles to each other (Figs. 53 to 60 inclusive). Thus in one division whole chromosomes are separated and in the other, each of these is divided transversely. Therefore no equational division occurs; and this to some would be a fatal objection to parasynapsis as outlined above. Before such an objection can be taken seriously it must first be shown that the supposed difference between "quantitative" and "qualitative" divisions really exists. The theory rests upon the assumption of the arrangement of the chromomeres of each chromosome

in a linear series so that a division in a plane including the axis of a chromosome, halves each particle in it, while on the other hand a division at right angles to the axis separates whole chromosomes. Further discussion of this question need not be entered into here for it is evident that so long as nothing definite is known of the arrangement or significance of the component parts of a chromosome, the absence of the so-called quantitative division need not be considered of especial importance. Similar conditions in other forms may be of much wider occurrence than is at present supposed.

It is seen from this account that there does not seem to be a true synapsis or complete fusion of chromosomes in the formation of the spireme of the prophase of the first maturation division. The duality of the series is perfectly evident and the frequent instances where the components are more or less separated speaks for the absence of fusion. The only fusion that has occurred is in the disappearance of the transverse furrow which in synizesis divided each chromosome so as to produce twice the somatic number of segments (Figs. 50 and 51). The disappearance of this furrow is only temporary, for it reappears in each bivalent chromosome after the spireme has broken up into the reduced number of segments (Figs. 52 and 53).

Cases like Fig. 49 showing the first step in the re-formation of the spireme after the resting period, suggest very strongly that the biserial arrangement of the chromosomes on the parallel linin threads connected with the chromatin nucleus persists through the resting stage. The evidence for what has been called "prochromosomes" is not very marked; the chromatin being represented by irregular masses whose number could not be determined with any exactness. Evidence along this line has been urged by many workers in support of the conception that the chromosomes are permanent cell structures. Boveri ('04) in his stating that the chromosomes are individual elementary cell organisms "*die in der Zelle ihre selbständige Existenz führen,*" has perhaps gone to the extreme in developing the individuality hypothesis; and Rabl ('06) has expressed a similar view.

Harper ('05) has pointed out that it is somewhat questionable



whether Boveri is justified in combining the conception of the permanence of the chromosomes and the doctrine that they are individual or elementary organisms which lead a relatively independent existence in the cell; and furthermore that such a conclusion does not necessarily follow from permanence in number, form and position in the nucleus, any more than that the cytoplasm is an individual organization because it grows and divides. No more is gained in support of the hypothesis of chromosome individuality by regarding the chromosomes as elementary and relatively independent organisms which bear a symbiotic relationship to the cell, than that they are definite permanent parts of a cell mechanism having a permanent, but not necessarily independent existence in the cell.

More recently Boveri ('07, p. 229) has given a definition of chromosome individuality that is not quite so rigid. "Was durch den kurzen Ausdruck, 'Individualität der Chromosomen' bezeichnet werden soll, ist die Annahme, dass sich für jedes Chromosoma das in einem Kern eingegangen ist, irgend eine Art von Einheit im ruhenden Kern erhält, welche die Grund ist, dass aus diesem ruhenden Kern wieder genau ebensoviele Chromosomen hervorgehen, und dass diese Chromosomen überdies da, wo vorher verschiedene Grössen unterschiedbar waren, wieder in den gleichen Grössenverhältnissen auftreten und dass sie dort, wo sie vor der Kernbildung in charakterischer Weise orientiert waren, diese Orientierung bei ihrer Wiedererscheinung häufig in gleichen Weise darbieten." This definition illustrates the general tendency to depart from the older idea of the chromosomes being strictly automatic individuals.

It is this conception of *automatism* against which most of the criticism of the chromosome hypothesis has been directed. The observations of a number of investigators seems to show that the chromosomes are represented in the resting stage between cell divisions. Thus Bonnevie ('08) has shown that in rapidly dividing cells (cleavage stages of *Ascaris* and root tip of *Alium*), although the identity of the original chromosomes is lost in the resting nucleus after each mitosis, each new chromosome arises by a kind of endogenous formation from within and from the substance of its



predecessor. In this way the genetic continuity of the chromosomes is preserved in the resting stages, "Eine genetische Continuität der Chromosomen nacheinanderfolgenden Mitosen konnte in der von mir untersuchten Objekten teils sicher (*Alium*, *Amphiuma*) teils mit überwiegender Wahrscheinlichkeit (*Ascaris*) verfolgt werden. Es ging aber auch hervor, dass eine Identität der Chromsomen verschiedener Mitosen nicht existiert, sondern dass jedes Chromosom in einem früher existierenden Endogen entstanden ist, um wieder am Ende seines Lebens für die endogene Entstehung eines neuen Chromosoms die Grundlage zu bilden" (p. 54).

Overton ('09) claims that in the somatic nuclei of *Thalistrum purpurascens* and *Calycanthus floridas* the chromosomes are represented during the resting stage by definite, visible bodies; the pro-chromosomes, which are arranged in parallel pairs with apparent linin intervals. Prochromosomes are also present in the resting nuclei of the germ cells of these plants and *Richardia africana*, in exactly the same arrangement and form as in the somatic nuclei. The homologous parental elements are already associated in pairs when they enter the reconstruction stage of the germ nuclei.

The results of these two investigators will serve to indicate the increasing evidence in favor of permanence of the chromosomes, which is to be held distinct from the idea of automatism in these bodies.

In the spermatocyte of *L. signaticollis*, when the spireme of the synzesis stage enters the rest stage showing a definite arrangement of its segments, and later emerges from that stage showing the same arrangement of parts, it is obvious that the organization which is responsible for this configuration must have persisted in some form through the intervening rest period; even though the outlines of the chromosomes and spireme are temporarily lost to view. In other forms where the evidence for prochromosomes is relatively clear, the existence of this organization is more apparent; but even here the chromosomes undergo a kind of vacuolization or disintegration, leaving but a mere skeleton to indicate their presence. In other words, each chromosome on entering the

resting stage passes into a condition of almost complete disorganization from which it is able to recover its individuality and reappear in its characteristic form in the prophase of the first maturation division.

According to the strict individuality hypothesis these transformations are to be explained solely by the remarkable properties possessed by the chromosomes as individual units.

The results of the present study show that the nucleus and cytoplasm of the cell taken together constitute a unit; while the chromosomes are regarded as the expression of an organization of the protoplasm of which they are a part. The occurrence of the amitotic period in the germ cells of both sexes clearly shows that this organization is a property of the protoplasm as a whole; for it is well nigh impossible to conceive of the chromosomes as independent entities, when during this amitotic cycle no provision is made for their transmission from generation to generation. It might be argued that in amitosis the chromosomes are divided with as great precision as in mitosis; but actual observations do not warrant such an assumption.

One of the strongest arguments that have been used in favor of the individuality hypothesis is the fact that in many cases at least the size of the nucleus is dependent upon the number of chromosomes that it contains. Thus Boveri ('05-'07) states that in sea urchin larvæ the surface area is proportional to the number of chromosomes contained in the nucleus, and that nuclei possessing an abnormal number of chromosomes pass on the abnormality to the daughter nuclei. The general truth of this statement is clearly indicated in the primordial germ cells of the ovary and the testis of *L. signaticollis* where occasional abnormal mitotic figures occur which result in the production of abnormal daughter nuclei.

Is it necessary to use the individuality hypothesis to explain these phenomena? Could they not be as well explained by considering the chromosomes simply as a portion of protoplasm possessing a definite organization?

During the last two or three decades the chromosomes have received much attention and the results of the combined efforts of

a large number of workers have yielded some interesting and important data; but the theoretical deductions drawn from these data have not been in all cases entirely justifiable. Attention has been focussed on the chromosomes largely because they happened to be objects made conspicuous by the readiness with which they can be stained with certain dyes employed in cytological technique, while other factors in heredity have been overlooked simply because they are not expressed so clearly in a morphological form. The chromosomes certainly have their place in hereditary processes, but the results of the present investigation indicate that they are but the manifestation of properties which belong to the cell protoplasm as a whole.

#### IV. SUMMARY

##### *The Ovary*

The functional germ cells of the ovary and the nurse cells have a common origin from the primordial germ cells. The configuration of the latter in larval and pupal stages is such as to suggest an amitotic division period of short duration. The nuclei at this time are characterized by the presence of a basic staining nucleolus in various stages of division surrounded by a clear non-staining area.

The epithelial cells are somatic cells derived from the mesodermic somites. During the larval and pupal stages the terminal thread contributes epithelial cells to the egg chamber. In pupal stages, the two regions become separated by a structureless "limiting membrane" which develops first at the sides and finally becomes continuous all the way across the ovariole. In the earlier history of the ovary, the relation of the epithelial cells to the germ cells is such as to indicate a nutritive function for these cells, but it does not appear that they enter into the formation of the egg follicles. The view is here advanced that the latter structures arise from the cells of the ovariole stalk. In later stages the epithelial cells are concerned in forming the delicate membranes which enclose the cysts. The peritoneal sheath of the ovary,

especially in the region of the terminal thread, becomes invested with striated muscle fibers, the whole being inserted in the dorsal wall of the body.

There is no evidence of a chromosome basis for the differentiation of functional germ cells from nurse cells. On the other hand the production of a semi-fluid medium from the interaction of epithelial and ovariole stalk cells, at the time when the differentiation is occurring, suggests that it is an important factor in the process. Those of the primordial germ cells coming under its influence are enabled to develop into functional ova; the remainder become nurse cells.

Mitotic division figures disappear first among the germ cells of the ovariole at its lower proximal end, but they can be seen for some time afterward in the more distal part among the potential nurse cells. The persistence of the mitotic divisions in the latter instance suggests that the energy which is conserved in the functional germ cells for the needs of developmental processes is here being expended in cell divisions. The frequent occurrence of multipolar and abnormally large single spindles is also noted. Mitotic figures are never seen inside of cysts in the ovariole.

The chromatin of the nuclei of the nurse cells appears more highly granular than that of the germ cells; and this is the first obvious result or accompaniment of the process of differentiation of the nurse cells. Amitosis and formation of cysts takes place first among those nurse cells at the base of the chamber and adjacent to the functional ova. The contents of each cyst are the descendants of a single mother cell. At first, only the nucleus divides; the result being large polynucleated masses. Later the division extends to the cytoplasm, producing in some cases mononucleated masses morphologically equivalent to single cells. Amitosis is regarded as an indication of an intense metabolic activity involved in the differentiation of the nurse cells which demands the most rapid and expedient method of nuclear division. This is in accordance with the view taken that amitosis represents a more primitive and relatively simpler method of cell multiplication than mitosis.

The prolonged amitotic period brings on certain chemical

changes which are assumed to be due to the failure of the nuclear membrane to rupture at regular intervals and discharge materials of the nature of oxydases or oxidizing substances into the cytoplasm, and the result is a reversal in the usual staining reactions of nucleus and cytoplasm. This change is preceded by the expulsion from the nucleus into the cytoplasm of a basic staining granule which gradually undergoes solution and disappears.

In their later history some of the nurse cells pass through a cycle of chemical changes which originate in the center of the nucleus and spread toward the periphery, leaving as a final stage, an acid-staining central area surrounded by three concentric shells of alternating basic and acid-staining regions.

The nutrition of the ovum is derived entirely from the nurse cells by means of an "egg-string," which is a pseudopodium-like process of the cytoplasm of the egg, that is left behind as the ovum moves down the tube in the early part of the growth period, and the distal end of which comes into relation with the spaces between the nurse cells. This intercellular region from its appearance in sections has been likened to a system of ducts into which the nutritive material from the nurse cells is secreted, and whence it passes via the egg-strings into the eggs.

The nutritive stream consists of basic staining granules identical with those found in the cytoplasm of the nurse cells. At first these granules are found evenly distributed in all parts of the cytoplasm of the ovocyte, but later, a definite stream extends from the end of the nurse string to the nucleus. In still later stages the deposition of the yolk in the center of the egg causes a split in the food-stream, compelling the latter to take the form of an oval shell inside of which the yolk masses are embedded.

Attention is called to a rhythm of chemical changes in the development of the ovum. The cytoplasm of the primordial germ cells shows the presence of an acid-staining reticulum which contains basic-staining granules apparently identical with those of the food-stream. As yolk is formed these granules of the food-stream disappear in the central part of the egg, being found only in the superficial cytoplasm. Thus it appears that the basic-staining granules from the nurse cells through interaction with

the nucleus and cytoplasm are converted into the inert acid-staining yolk. After fertilization, the basic staining yolk nuclei react with the yolk and convert it from its inert condition into a form which can be readily assimilated by the living protoplasm of the blastoderm.

Likewise, the nucleus passes through an interesting series of changes. Beginning with the end of the division period, the ground work of the nucleus is a clear, homogeneous, non-staining substance, in which a basic-staining nucleolus and chromosomes are embedded. As the growth proceeds, the chromosomes lose their sharp outline and gradually take on a filmy form in which they stain deeply with acid dye. Finally in the nearly mature egg, all of the nuclear contents appear granular and takes the acid stain, only the nucleoli which have increased greatly in number take the basic stain.

### *The Testis*

In the larva each lobe of the testis is of a cylindrical form resembling a single ovariole of the ovary. As in the latter both epithelial and germ cells can be readily identified. In the ensuing stages the increase in size takes place principally at right angles to the axis, but not equally in all directions, being inhibited at regular intervals which mark the spaces between the radiating follicles. The original apex is represented in the adult organ by a cap-like lobe which lies just opposite the aperture of the sperm duct.

The epithelial investment, at first loosely applied, in late pupa stages enlarges between the follicles and produces the appearance characteristic of the adult in which the follicles are separated from each other by a thick layer of epithelial cells.

Amitotic division figures of the same type observed in the germ cells of the ovary can be seen in the germ cells of the testis of the larva, the pupa and even the adult in the cap-shaped region. All of the germ cells pass through the amitotic cycle which commences at the beginning of cyst formation and persists for a number of cell generations. These cells later divide mitotically.

Areas of degeneration are to be seen in all stages of the testis

beginning in the larva and extending through the adult. The process commences with accumulation of epithelial cells in the region representing the lumen of the general cavity of the testis continuous with that of the sperm duct of the adult. The cells involved fragment, producing irregular masses which stain deeply with the basic dyes. As the degeneration proceeds the area contracts until in the adult it is represented by a concavo-convex disc-shaped region marked off from the neighboring parts by a connective tissue capsule. The process is regarded as a method of providing nutritive material either for the spermatozoa or for the germ cells during the maturation period. Thus the cells that undergo degeneration are looked upon as the nurse cells of the testis.

#### *Amitosis*

The occurrence of amitosis at corresponding stages in the germ cells of both sexes is believed to be due to a periodic fluctuation in the nutritive supply of the cells brought about by a stimulus to a rapid cell division which causes a temporary derangement in the normal metabolism. In the ovary the disturbance is merely transient; but in the testis it is more prolonged for the reason apparently that it is here involved in the formation of cysts, a process that in the species under consideration is always accompanied by rapid cell multiplication.

In the nurse cells the initial cause of amitosis is probably the same; but in this instance it is carried to an extreme, so that a permanent change in metabolism occurs.

Amitosis and mitosis are believed to stand for the extremes of a continuous series; the different configurations of the division figures being due to the different types of metabolism represented.

#### *The Chromosomes*

The first spermatocyte spindle at metaphase, shows the presence of sixteen bivalent chromosomes that divide in this division, and a bivalent V-shaped body, made up of equal parts, that does not divide but passes entire to one pole.

In the second division all of the chromosomes including the bi-partite body divide. The latter probably divides longitudinally so that two kinds of spermatids result, containing sixteen and eighteen chromosomes respectively.

The odd body is a basic-staining nucleolus that makes its appearance in the synizesis stage when the other chromosomes are in the form of a doubly segmented spireme whose halves may be more or less separated.

In the rest stage following, the nucleolus persists as a deeply basic-staining bi-partite body while the remaining chromosomes are represented by faintly staining clumps of matter distributed on an irregular reticulum.

The first step in the formation of the spireme of the ensuing prophase consists in the appearance of two parallel strands of linin extending from the nucleolus. These apparently represent the framework of the spireme not only of this stage but of the preceding synizesis.

When the spireme appears, it is composed of a thickened double thread whose halves are more or less closely applied to one another, each of which contains sixteen segments. The nucleolus which is attached to the nuclear membrane forms an additional member in the series.

After the spireme has broken up the sixteen bivalent chromosomes proper immediately shows signs of a second groove at right angles to the first producing tetrad-like bodies. The bi-partite chromatin nucleolus of the resting stage never shows such evidence of a second division.

In the ovocyte at a corresponding time there is a similar synizesis stage and bi-partite chromatin nucleolus. Instead of passing directly into a rest stage as in the male, the chromatin knot becomes disentangled producing the characteristic "spireme" stage which persists for quite a while.

In *L. decemlineata* an odd body appears in the first spermatocyte spindles, but in this case it is composed of two unequal parts. Its behaviour in the maturation divisions is believed to be the same as that described for the homologous body in *signaticollis*. Synizesis stages of the ovocyte of *decemlineata* show a bi-partite nucleolus also composed of unequal parts.



It is assumed that in both species the odd body represents two conjugated somatic chromosomes.

There is much evidence to show that the spireme is formed in the post-telophase stages of the last pre-maturation division by the arrangement of the chromosomes into two parental series which become closely applied to each other (parasynapsis) producing in this way the double spireme characteristic of synizesis.

The evidence from observation does not justify the view that the chromosomes are independant individual units of a lower order than the cell. On the other hand, they are to be regarded as permanent cell structures in so far as they express a definite organization of the protoplasm. During amitosis this organization is assumed to persist, although the chromosomes which represent it in mitosis do not come to expression. This is believed to be due to differences in cell metabolism in the two cases.

Accepted by The Wistar Institute of Anatomy and Biology, February 10, 1910. Printed August 3, 1910.

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#### ABBREVIATIONS.

*a. c.*—apical cell

*am.*—amitosis

*e. c.*—egg cell

*ep. c.*—epithelial cell

*gr.*—granule

*g. c.*—germ cell

*l. m.*—limiting membrane

*m.*—mitotic figure

*n. c.*—nurse cell

*n, stm.*—nutritive stream

*n. str.*—nutritive string (egg string)

*p. sh.*—peritoneal sheath

*o. st. c.*—ovariole stalk cell

*t. pr.*—tunica propria

*x.*—chromatin nucleolus of the resting stage.

The figures in the plates are camera drawings made at stage level with the following combinations of Zeiss apochromatic objectives and compensating eyepieces.

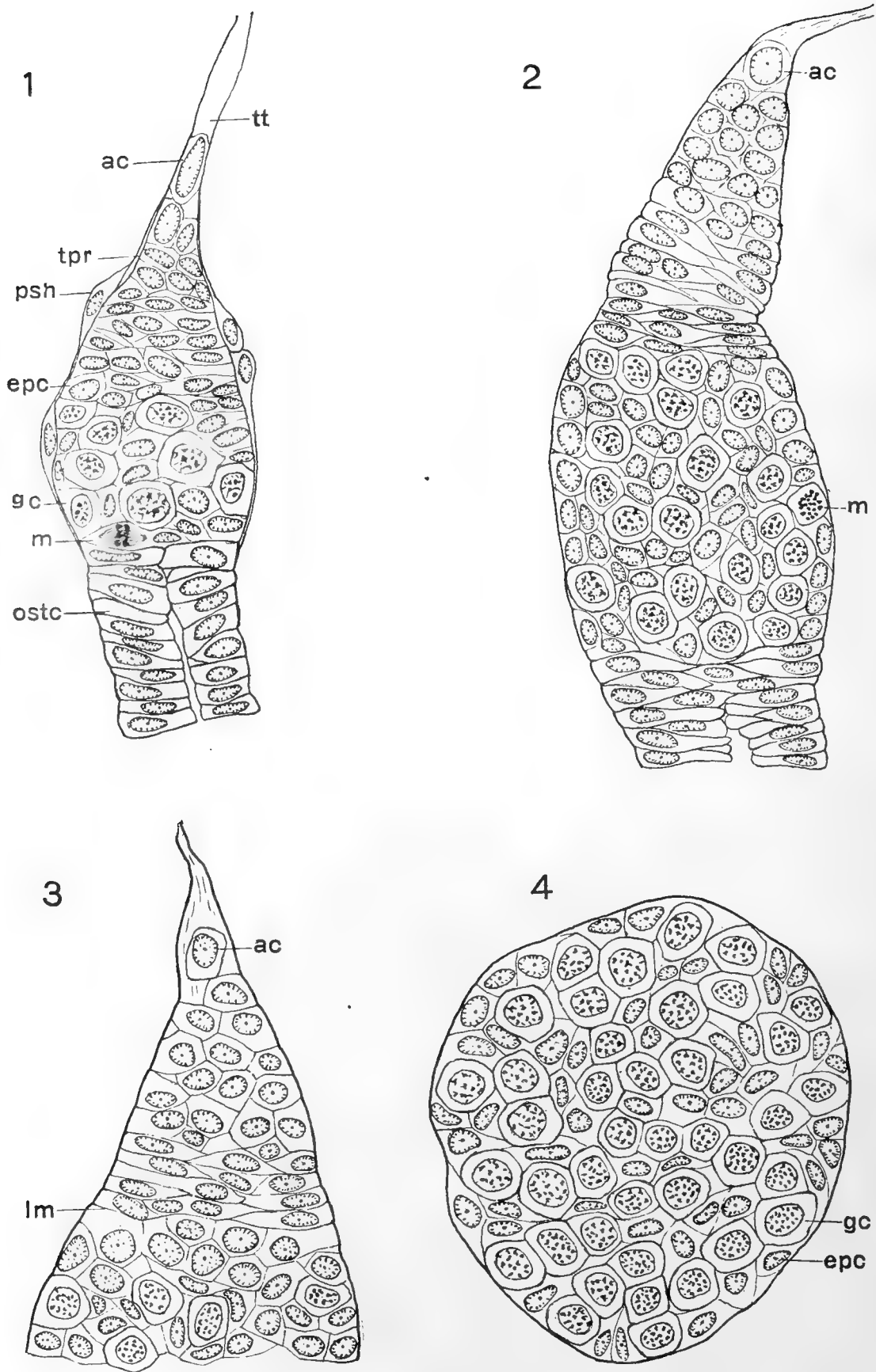
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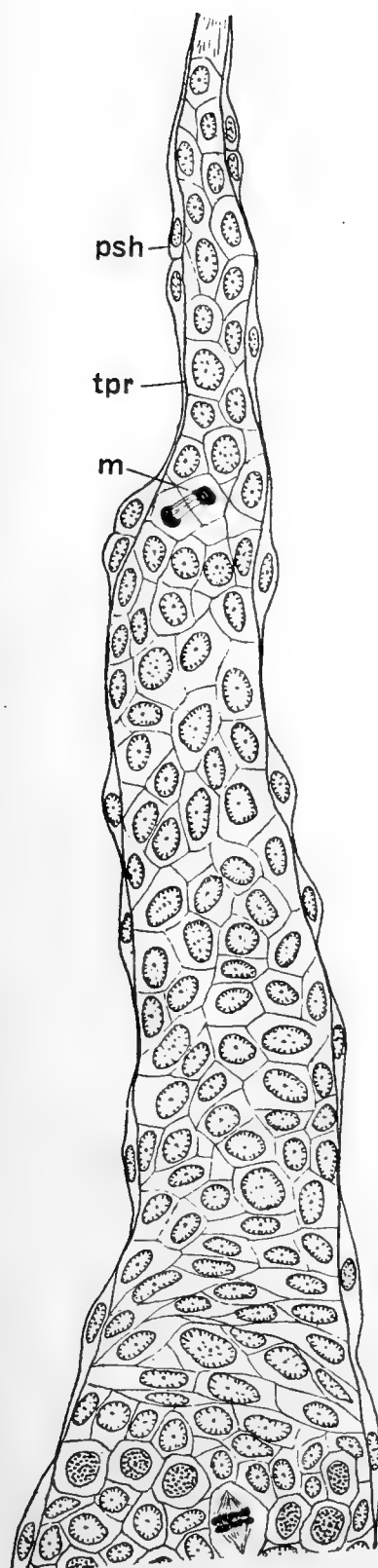
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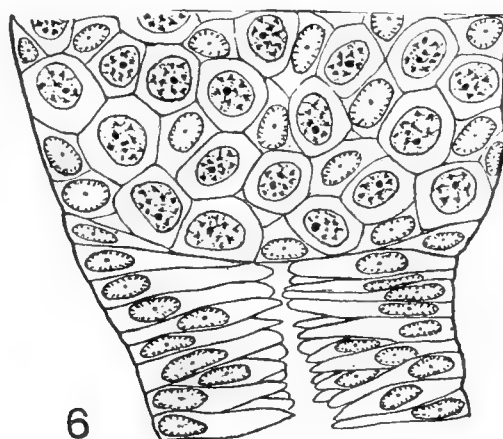
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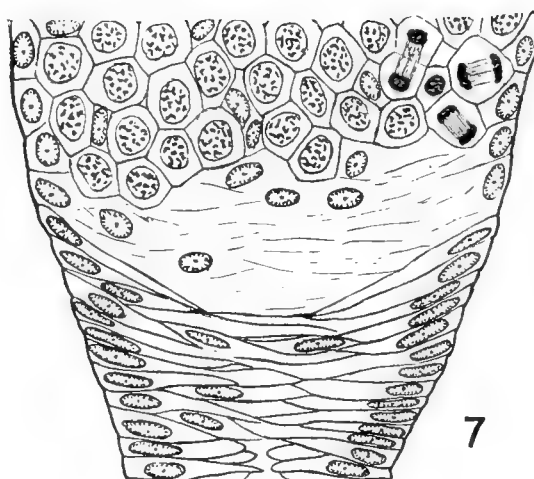




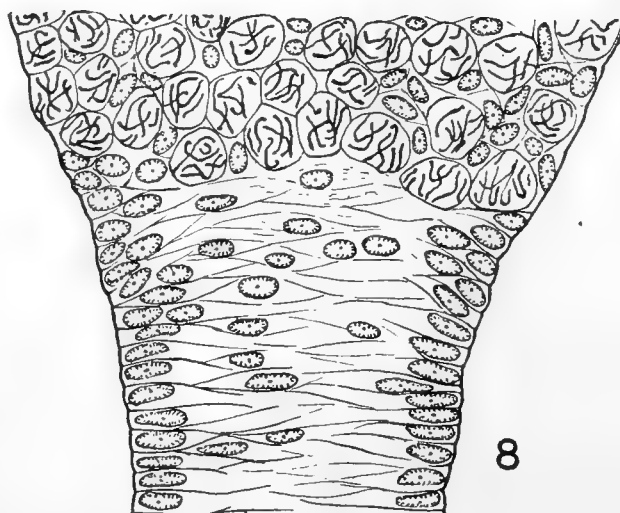
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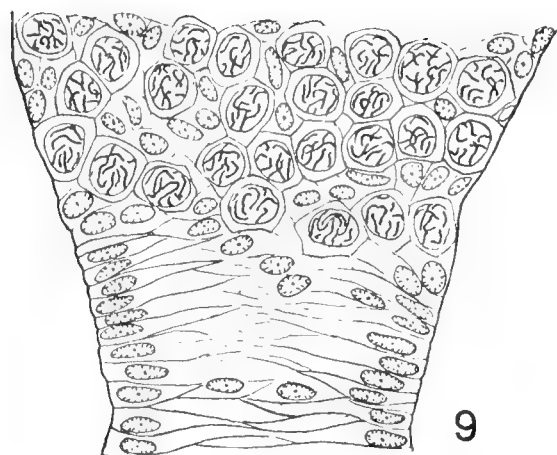
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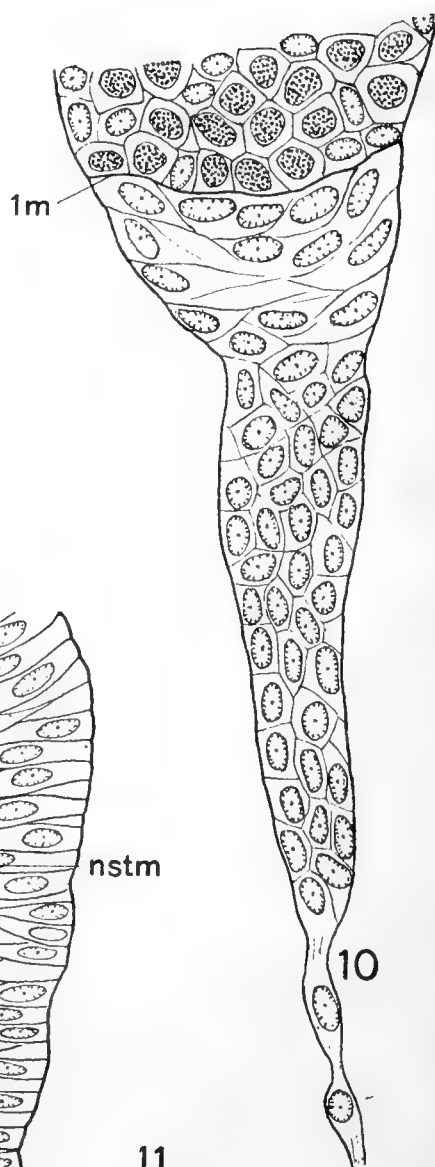
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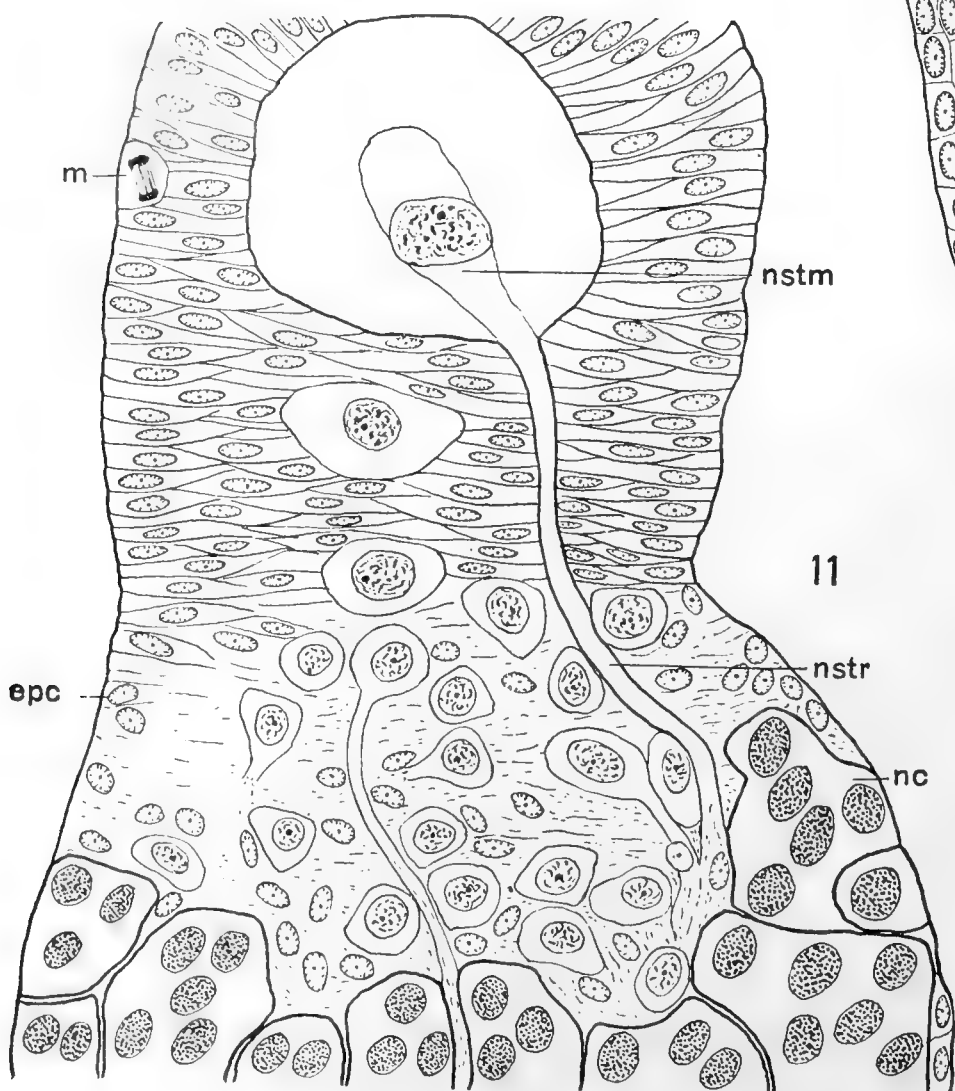


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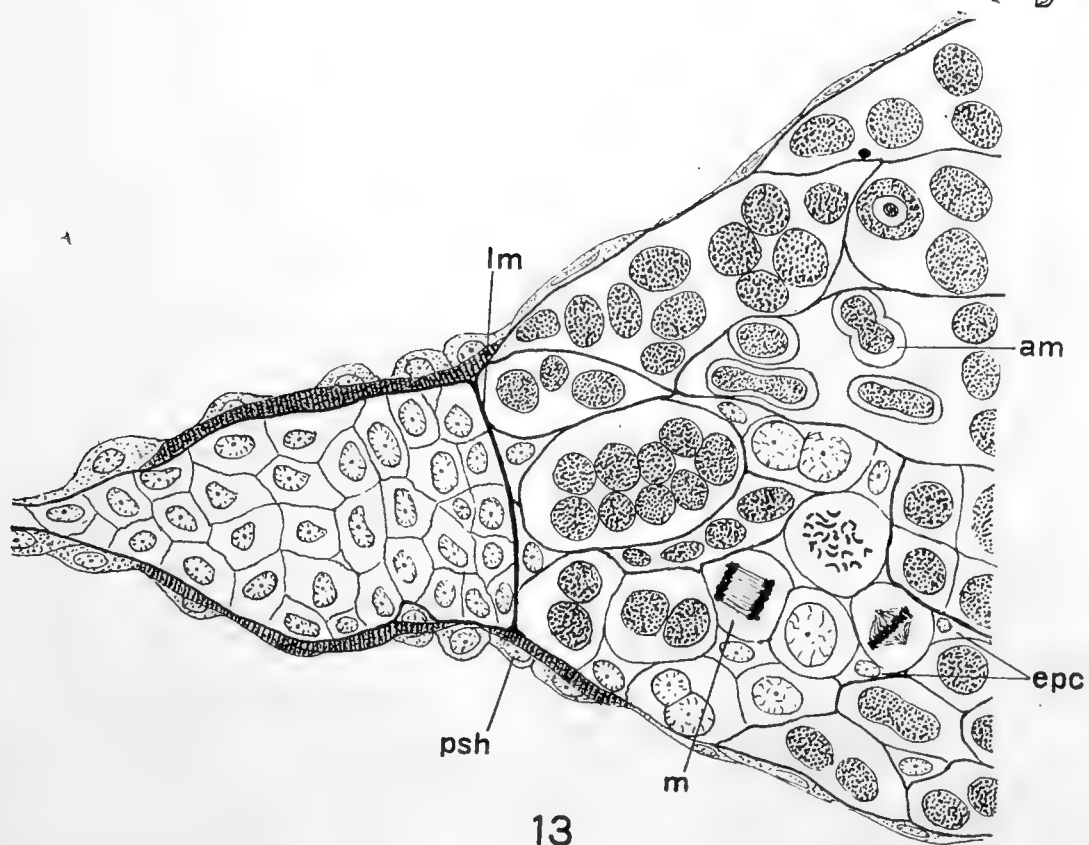
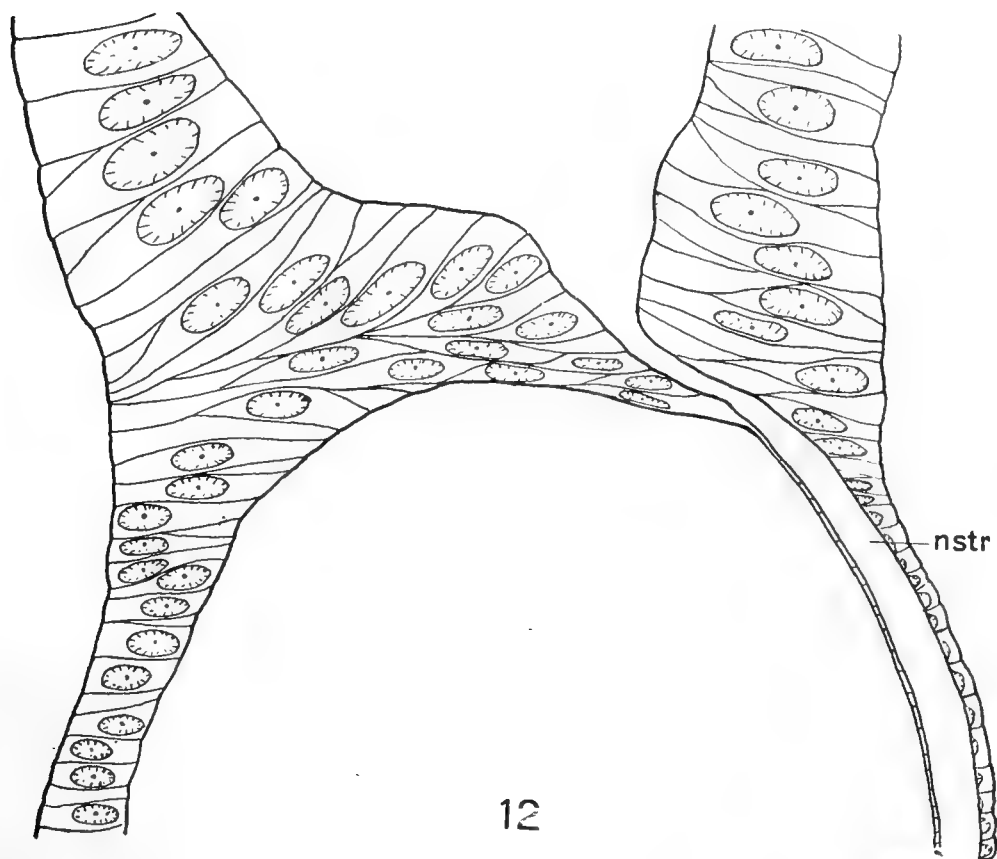
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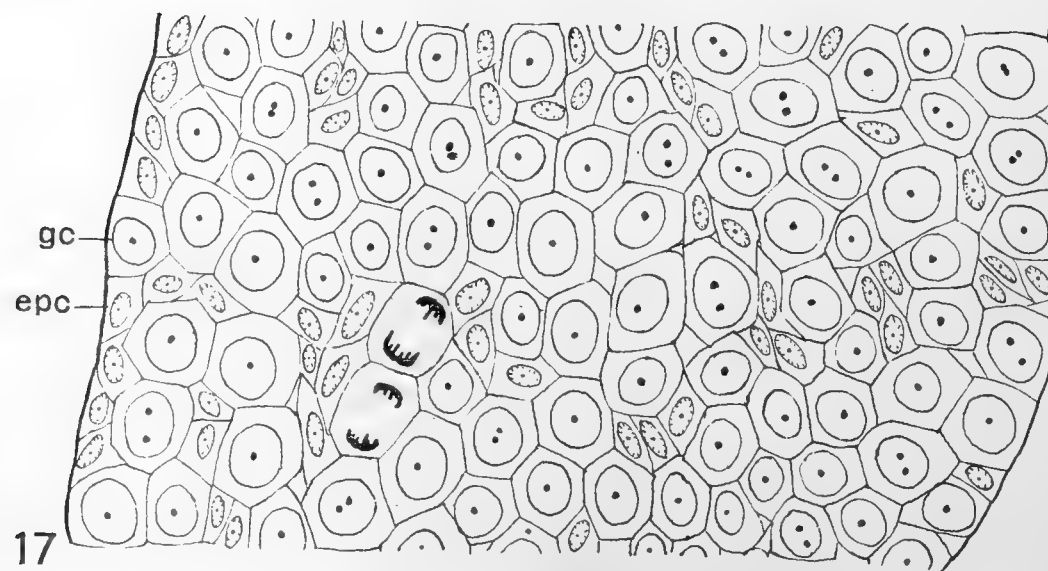
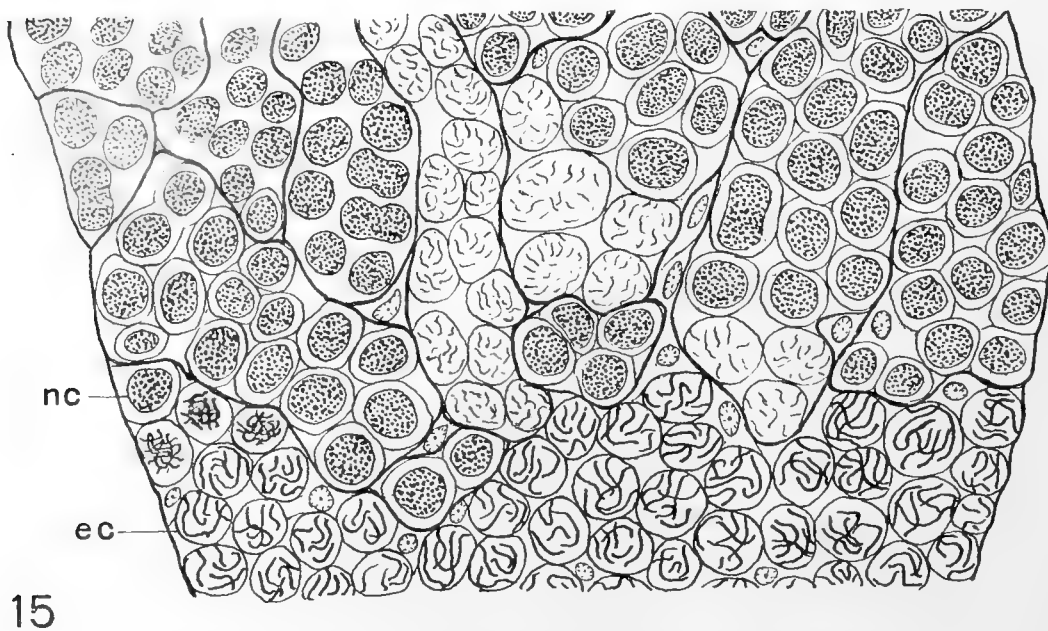
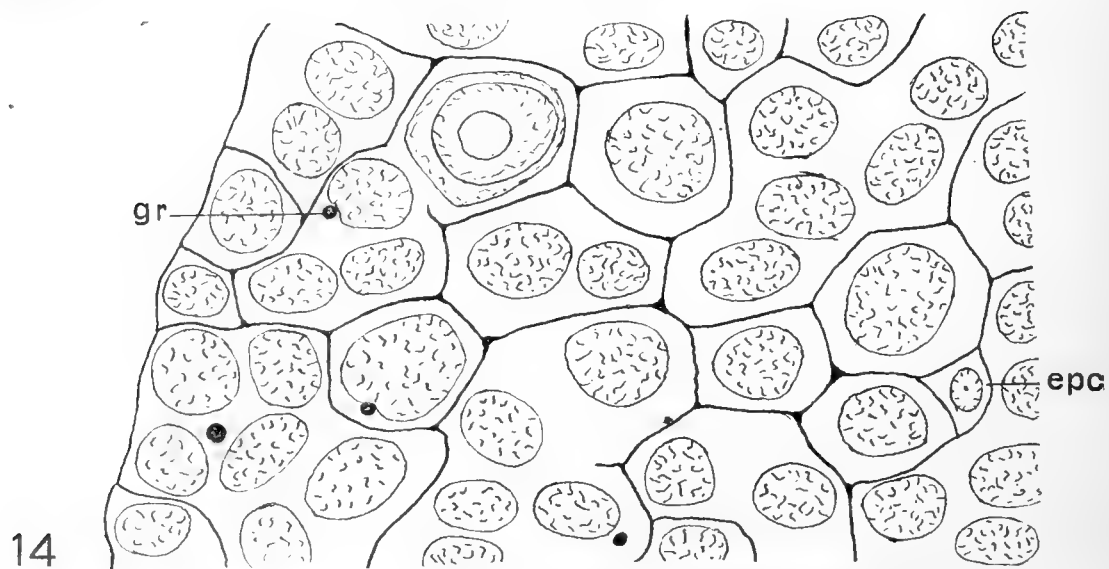
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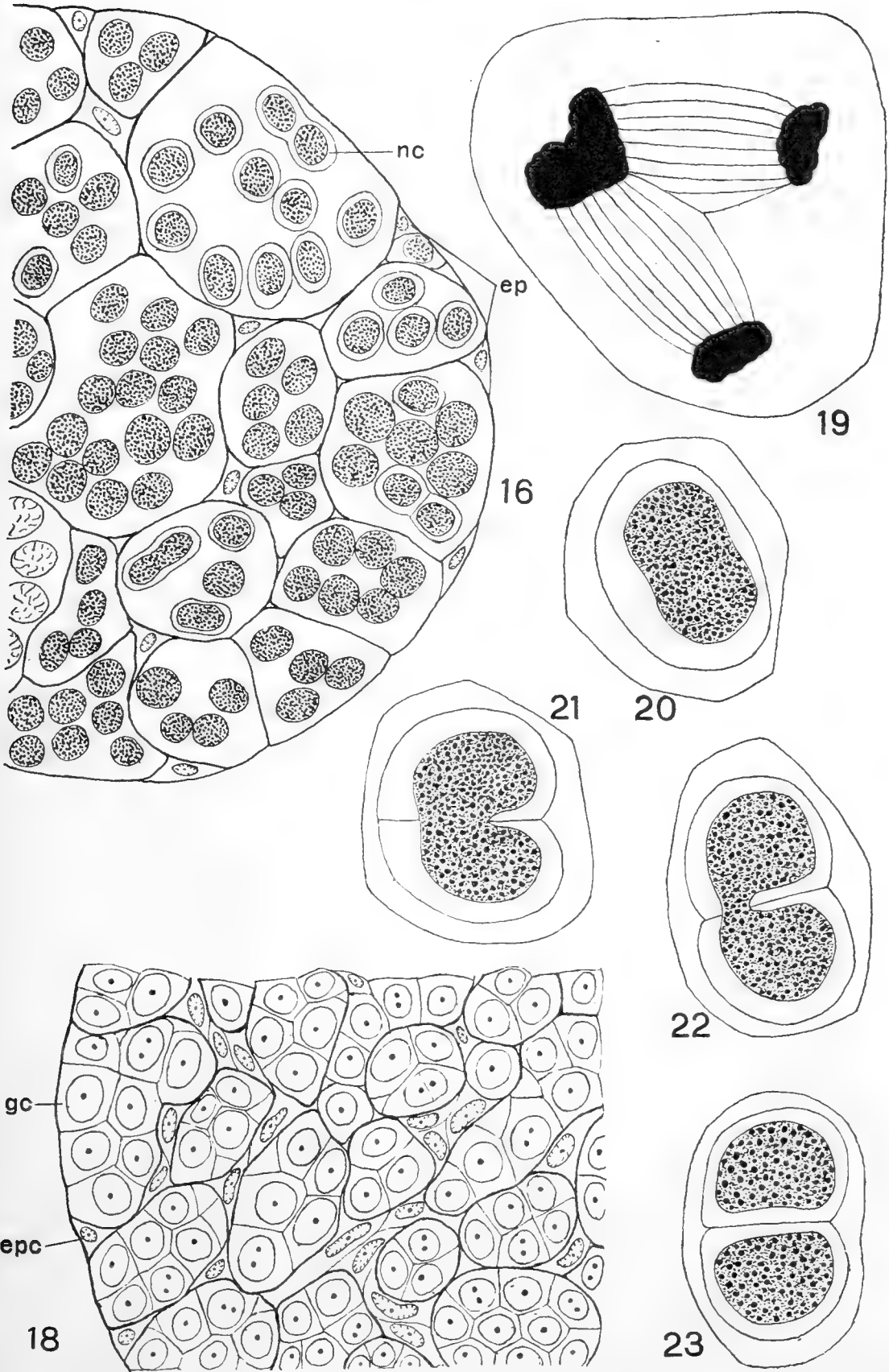
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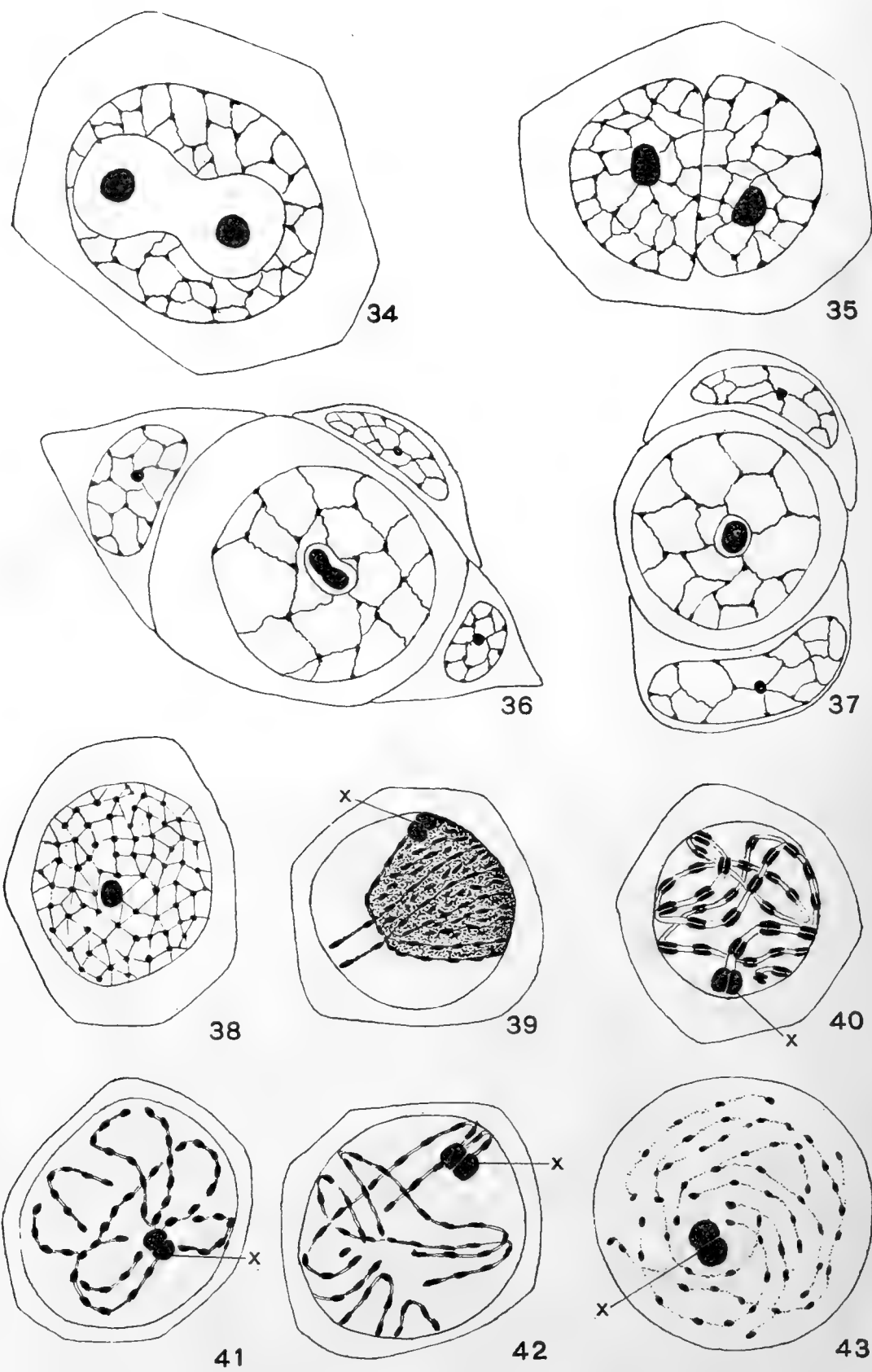
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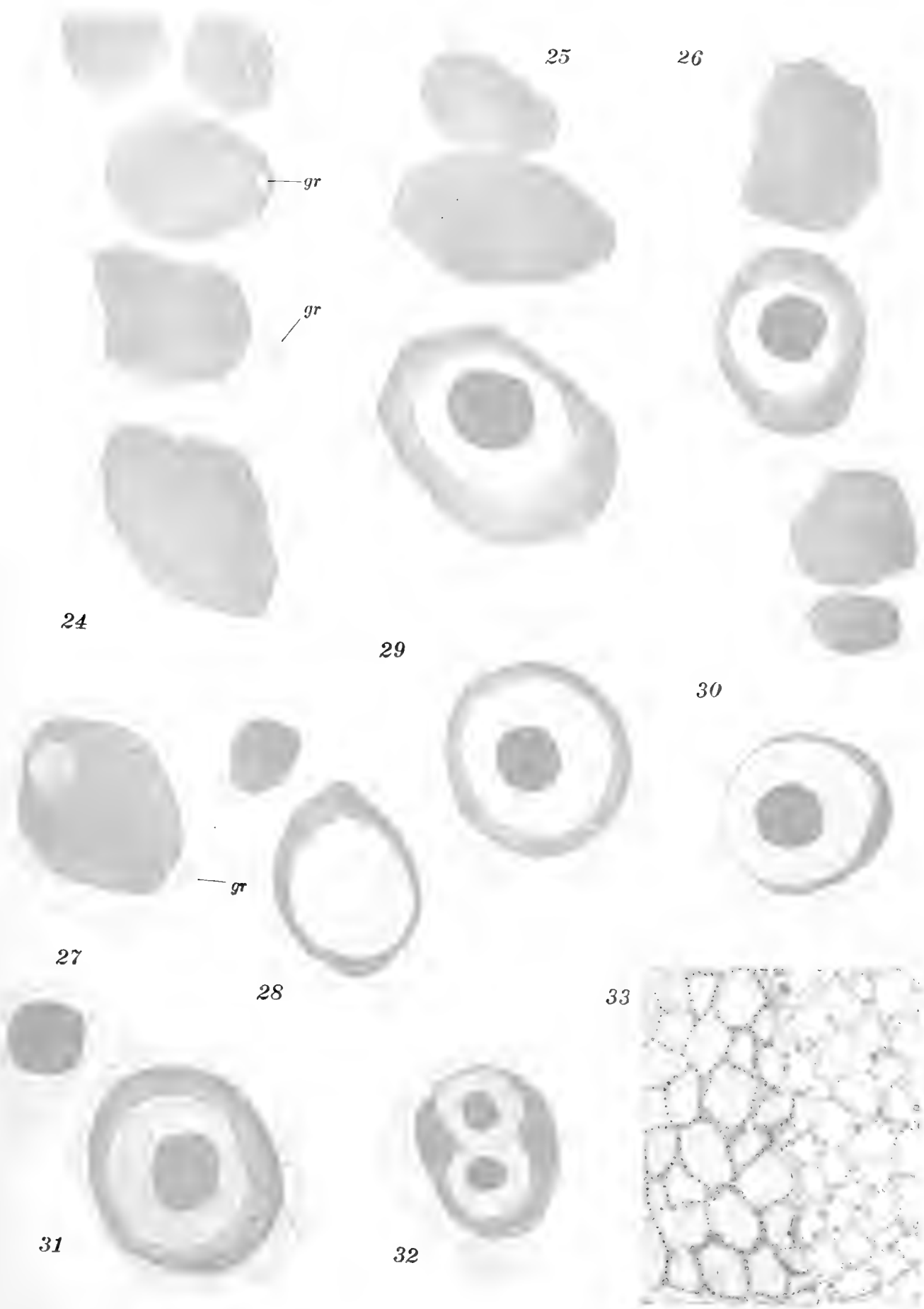








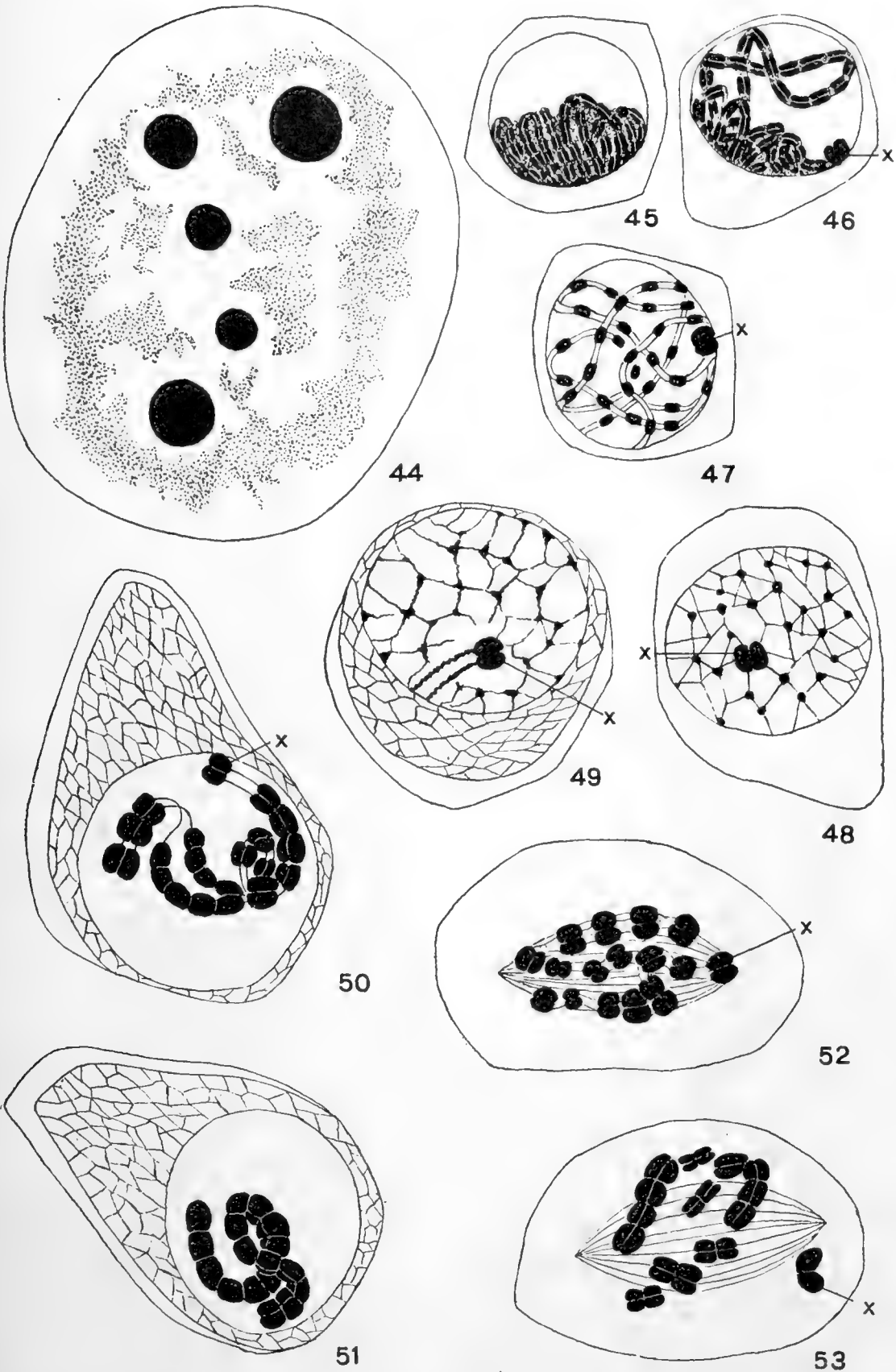


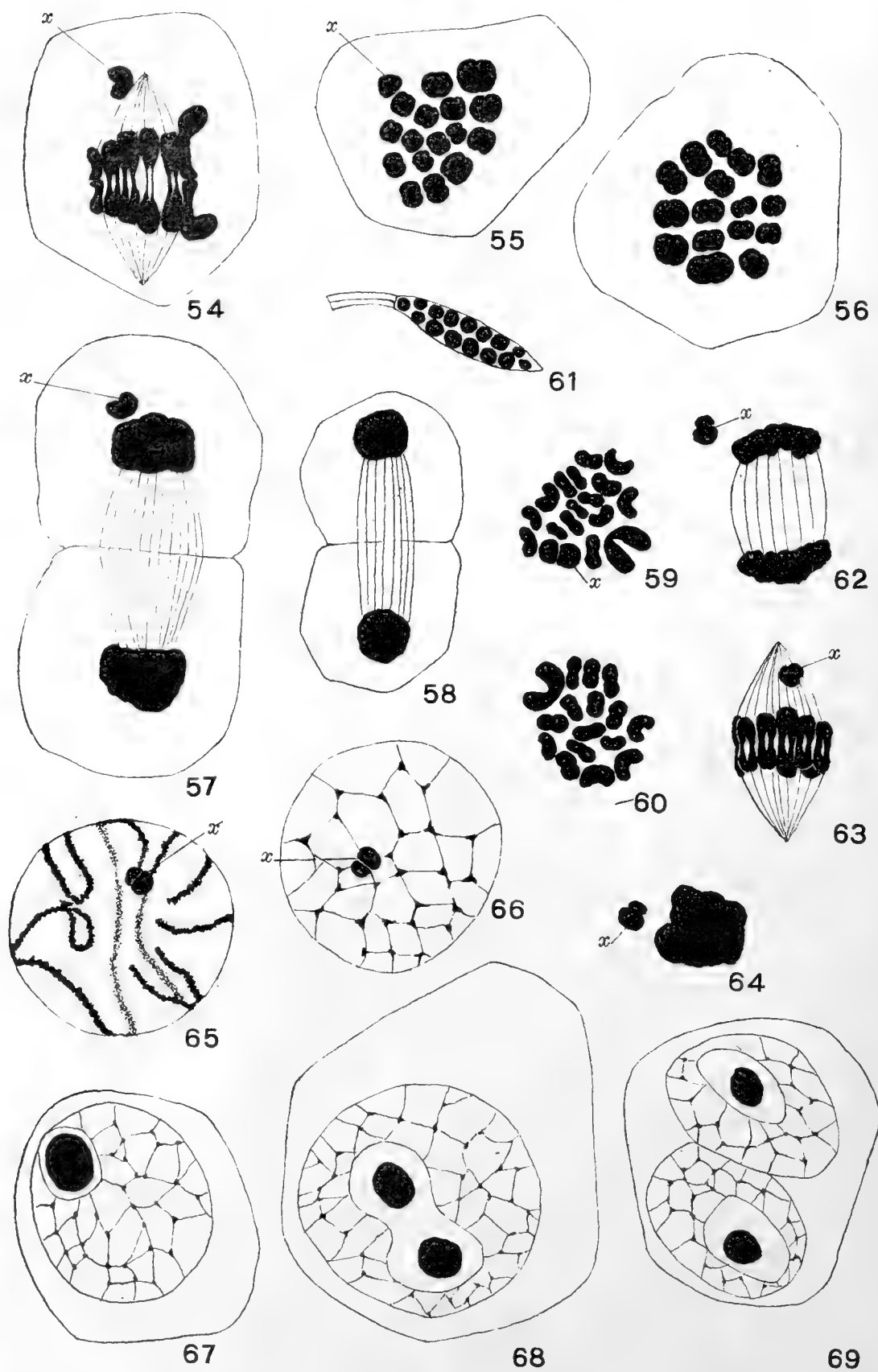


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# STUDIES IN THE DEVELOPMENT OF SCYPHOMEDUSÆ

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WITH FORTY-NINE FIGURES

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## INTRODUCTION

Several years ago the senior author began a more or less detailed study of the life history and development of *Cyanea arctica*, devoting to the subject both laboratory research, and such attention to distribution and open sea habits as was afforded by a cruise of several days to the Gulf Stream on the schooner *Grampus*, of the Fisheries Bureau.

The various features of larval development were followed almost continuously during the entire summer, a brief preliminary report of which was published in the *American Naturalist* (1902 b, pp. 555-559). Unfortunately the material obtained at this time was deficient in the earlier stages of egg development, especially those relating to maturation, fertilization and early cleavage. Attempts were repeatedly made at subsequent times to secure the desired material for completing the problem, but with only partial success; and it was not till the spring of 1908, in April, that the junior author fortunately succeeded in obtaining a fairly adequate supply for certain of the missing phases. He has accordingly, been associated in the present work of carrying forward the problem to a degree of completion which would have otherwise been more or less impracticable, and is chiefly responsible for the cytological part of the paper.

#### MATERIAL AND METHODS

The earlier material was obtained in July, 1901, from a few specimens of medusæ which had apparently drifted into the harbor at Woods Hole, and were bearing ripe gonads, or rather embryos in early stages of development. The occurrence of the specimens at this time, and in this condition, would seem to be more or less unusual, the usual breeding season for this medusa being in April and early May in this region. But as will be mentioned in another connection, there are known to be many variations and exceptions to this rule. As already intimated, the material was quite abundant so far as certain stages were concerned. Unfortunately, however, the methods employed in fixation of the material, chiefly picro-sulphuric and picro-nitric, reagents then much in vogue, were found to be almost worthless so far as these eggs were concerned, and as a consequence no satisfactory cytological results were obtained. Later material was fixed in various ways several of which proved most excellent, among which Bouin's fluid and Zenker's seemed to give the most uniform type of fixation, and gave no subsequent difficulty in staining. We also obtained during the current summer at Harpswell several ripe

specimens of *Aurelia* from which some excellent material was obtained. It was fixed by the same methods as just mentioned. In this case care was taken to secure early stages by removing portions of the ovaries and fixing in mass in Bouin's fluid and this later yielded some very desirable stages which would otherwise have been lacking.

#### GENERAL ACCOUNT

Comparatively little is known as to details of life history and habits of Scyphomedusæ. This is not to be taken as implying lack of knowledge as to many phases of developmental history in certain species. For example, it is well known that in the case of *Aurelia* and *Cyanea* there is a perfectly clear history from planula to ephyra, involving the intermediate phases of the polyp and its later strobilation to give birth to the ephyra. Further it is also well known that there is direct metamorphosis of the ephyra into the young medusa. But there are matters of detail as to the time involved in certain stages, which are yet uncertain. For example, it was pointed out in the earlier paper (1902 b,) that in some instances planulæ were early transformed into polyps, and that these in turn early strobilated and gave birth to ephyrae, while others continued in these respective stages for a relatively long time. Then too, it is a matter of uncertainty as to the details of the life habits of the adult medusæ; their length of life, mode of life, rate of growth, etc. In general it seems fairly certain that the spawning season is in early spring, March to May, but with notable exceptions as pointed out in an earlier connection. Hence it may be assumed that while the earlier season is predominant in our latitude it is not restricted to this time. In the case of *Aurelia* the spawning season is chiefly mid or later summer, but also with probable exceptions as in the other case, a few specimens breeding in early spring along with *Cyanea*.

Now, as to the further features of life history, range of depth, length of life, etc., there is less certainty. It may be stated as a general fact that the entire life cycle falls within the period of one

year, often much less. This is especially the case with *Cyanea*. As shown in the previous paper the entire time from the liberation of the planula to the free-swimming ephyra may be as brief as fifteen to twenty days. The growth of the young medusæ is rapid, and sexual maturity may be reached within a few weeks, or perhaps months. Just what may be the range of habits in the case of *Cyanea*, whether it is to any considerable degree given to inhabiting the deeper waters at any definite period of life, is a matter of uncertainty. There is little evidence, such as may be afforded by the dredge or trawl, in support of such a view; and on the other hand it seems almost certain that they are not predominantly pelagic. Conditions of weather have much to do with this feature. A rough surface invariably drives them downward, and calm weather is the occasion of a reverse movement. And this may be assumed as more or less the case with many other medusæ, as well as with other organisms of similar habits of life, such as ctenophores, copepods, etc.

Sofar as we have been able to observe there is no distinguishable influence of light or darkness in the movements of these medusæ. we have found them under all conditions of light—early morning, late twilight, the full glare of mid-day sun, indeed so common is *Cyanea* in this full sun glare that it is commonly designated by fishermen as the 'sun scald.'

#### BREEDING HABITS

The more or less sudden appearance of medusæ in a given locality, which has often been observed and remarked upon, has been regarded by many as due to a breeding instinct which leads them at such times to seek each other. The elder Agassiz was quite explicit on this point, stating that "at the time of spawning toward the end of July or beginning of August they may be seen gathering and clustering near together. That at this time they seek each other is unquestionable. I witnessed once, in front of my house at Nahant, a shoal of them, which was evidently in the act of spawning. Myriads of specimens had clustered together so closely that they formed an unbroken mass between which an

oar could not be thrust without hitting many at one blow. That they were actually spawning was ascertained by raising specimens from the water, when sperm was seen streaming freely from the appendages of the lower surface, and eggs flowing along the channel of their arms. It was about sunset and the closing night prevented me from ascertaining how long they remained together. The next day they were scattered by the wind, and a few days afterward immense numbers were found stranded upon the rocks and long sand-beach at Nahant." (1862, p. 76). A. Agassiz has also given expression to similar views on this point, though without definite facts other than the mere observation of the congregating of medusæ at periodic times. (1865, p. 46).

Much more explicit is a recent reference of Conklin (1908, pp. 155-156), to the spawning of *Linerges mercurius*. In the case of this medusa there seems little doubt as to the facts given by Conklin and their bearing upon the matter under consideration. Something of a similar character is also known among Hydromedusæ. The senior author has, in connection with the account of the development of *Pennaria*, made clear the intimate correlation of the spawning habits. He has also referred to the interesting occurrence of swarms of *Rhegmatodes* at certain times. But the latter have not apparently been associated directly with the breeding instinct, since they included specimens of all ages and conditions of maturity. He has also expressed serious doubt (1904, p. 26), of this interpretation as applicable to all cases. That the view of Agassiz cited above *may* find an *occasional* warrant need not be denied; but that it is *general*, or at all *frequent* we do not believe. In many years of observation little evidence has been found to sustain the view. Furthermore, the fact that isolated specimens of both *Aurelia* and *Cyanea* have been taken, males and females bearing ripe gonads, the latter in various stages of maturity and with eggs in various stages of development, would further support it. Indeed, in the case of *Cyanea*, it is rather rare to find any considerable numbers together. The first collection included but three or four specimens, including both sexes. Again in the case of *Aurelia*, which we collected in large numbers about the first of August, there was no massing of numbers at any time.



Collections were made at all hours of the day in various localities, and while the sexes were seemingly in approximately equal numbers there was no evidence of "seeking each other," such as Agassiz asserts. Still a further fact of even greater significance remains to be noted, namely, that in both Aurelia and Cyanea spawning is not a *single*, or *spasmodic* process, but one continuing during several days. One finds on a given specimen embryos in all stages of growth, from blastulæ to planulæ, and eggs in all stages of cleavage, and at the same time the ovaries loaded with eggs in various stages of growth, from oögonia to ova in maturation. It is difficult to correlate this condition with the assumption of any sudden, single act of spawning.

*Egg-laying*—This feature has been referred to incidentally in a previous section. It only remains to call attention to a few points not already mentioned. It is possible to distinguish the males and females of Aurelia when sexually mature and bearing gonads: the male organs being milky-white, while in the female they are pale-pinkish or purplish, the eggs having these tints when viewed in any considerable mass. This is less marked in Cyanea, in which both sexes have whitish or cream-colored gonads.

So far as our observations go there is no definite time of day at which egg laying takes place. This is seemingly in sharp contrast with what is known in many other medusæ. Conklin (loc. cit. p. 157), finds in the case of Linerges that this occurs "about 8 a. m., and at no other period of the day." At this time he finds that for a short time "a perfect epidemic of egg-laying takes place, after which no other eggs are laid till the following day." We have referred to a similar condition in Pennaria, though in this case it occurs in the evening, just about the twilight. The account of Professor Agassiz, previously cited, would indicate something similar in Aurelia. But this must be considered very doubtful. We have collected Aurelia at all hours of the day, and the senior author has kept females in the aquarium for days in succession, but in no case has there been apparent any such exhibition as would warrant the assumption that egg-laying in either of these medusæ takes place at any definite time. We were the more particular upon this point in collecting eggs of Aurelia, since it was desirable to

obtain ova in early maturation and cleavage. This it was not possible to do in any quantity, egg-laying appearing to be a more or less continuous process during all times of day, and for a number of days in succession. Another feature will make this more evident. In both *Aurelia* and *Cyanea* the eggs are not definitely discharged from the female; but after dehiscence from the ovary into the gastric cavity, where apparently fertilization takes place, they are 'nursed' in pocket-like folds of the oral arms for some time or until they are well-developed planulæ. Hence one finds on attempting to collect eggs that he gets all conditions of development at the same time, though in the immediate proximity of the mouth there will be a preponderance of cleavage stages and early blastulæ. That egg-laying is not a single process, as shown above, is evident in that one finds specimens with the gonads in various stages of depletion. Furthermore, in a study of the histology of the gonads one finds eggs in all stages of growth, a fact incompatible with the assumption of the shedding of the entire crop at a single time.

The eggs, when they escape from the ovaries, have already gone through the process of maturation. This would seem to take place just prior to the rupture of the follicular membrane by the egg, as will be shown in a later connection.

Following the liberation of the sexual products, and in the case of the females the final escape of the larvæ, there seems to be a rather rapid decline in the vigor and activity of *Aurelia* and early death ensues. Many specimens may be found drifting along shore lines and in harbors, and, if there be wind or tide driving them shoreward, they become stranded and rapidly disintegrate. An examination of many such during the past summer showed that in most cases such specimens were dead or dying when they came ashore. The case of *Cyanea* seems somewhat different. This medusa seems to live for considerable time after passing the spawning period. As stated before, the breeding season is usually in spring. But it is not unusual to find many specimens during early and late summer swimming freely in the usual manner. It is, however, rare to find such specimens bearing genital products. In many specimens collected in Casco Bay during the past summer not one was found with gonads.

## HISTORICAL

It was found by Kowalevsky (1873) that *Cassiopea borbonica* and *Aurelia aurita* went through regular cleavage, and a blastula with a relatively small cavity was formed. The entoderm was formed through a small invagination of the blastula wall, and was soon entirely separated from the ectoderm, a relatively larger cavity remaining between the two layers. Claus (1878) stated that *Chrysaora* passed its entire embryonic development within the ovary, emerging as a planula. The egg cells originated in the germinal epithelium and were covered by a follicle developed from this layer. The unequal cleavage, he believed, began while the eggs were still small and continued during the growing period, resulting in a blastula with shorter cells at one pole. From this region an ingrowth of cells took place and the entoderm was formed from these, the cleavage cavity being obliterated and the blastopore completely closed.

Haeckel (1881) studying *Aurelia aurita*, agreed with Claus (*Chrysaora*) that the first cleavage was not quite equal, and he referred to a differentiation of the smaller (animal) and larger (vegetal) blastomere, a difference believed to be retained for a considerable time. This inequality disappeared in later cleavage so that the blastula was composed of a large number of equal cells, enclosing a large cavity. The gastrula was formed by an invagination of one side of the blastula, the blastopore closing, but later the larval mouth ('nachmund') breaking through in the same place. As variations from the above processes he found that the cleavage might be quite unequal; that the invagination might be incomplete, the archenteron limited to about one third of the cleavage cavity, the remainder being filled with a jelly-like substance. When the gastrula was formed thus it usually transformed directly into an ephyra, skipping the planula, scyphostoma and strobila stages; or the blastopore of the completely invaginated gastrula remained open and the embryo settled down as an actinula with several tentacles, thus omitting the free-swimming planula stage. He also agreed with some earlier authors that the planula might bud or divide into several planulæ each of which formed a scyphostoma.

Claus (1883) was the next to study *Aurelia aurita*. He called attention to the fact, known to early observers, that the eggs pass from the ovaries into the gastric cavity and from there into the folds of the manubrium where development takes place. Haeckel was incorrect in thinking the first cleavage unequal and especially in the differentiation of the two blastomeres, since, as Claus stated, the first cleavage furrow passing through the animal pole can not divide the egg into an animal and vegetal blastomere. The second cleavage Claus found to be meridional and the third equatorial. Up to this time cleavage was equal but thereafter might be unequal and form a blastula with shorter cells at one pole. As he found that the cleavage cavity, present as early as the 8-cell stage, increased only slightly and consequently remained small, he believed Haeckel was mistaken about the large cavity and wide invagination. Because of the small size of the invagination the cells were crowded together and resembled very much the appearance of the cell mass formed by ingression in *Aequorea*, but he was able to show that a real invagination had taken place. In *Chrysaora* Claus found essentially the same process of cleavage except for the smaller egg, the development within the ovary and the very large cleavage cavity. An ingrowth of cells from the thicker portion of the blastula wall formed a solid plug but with cells arranged in two rows, so that the process was quite close to an invagination. As the archenteron grew the ectoderm also extended, and for a long time there remained a space between the ectoderm and entoderm.

In *Lucernaria* Kowalevsky (1884) found a regular and equal cleavage, the first and second furrows meridional, the third equatorial. No cleavage cavity was formed, segmentation resulting in a solid mass of cells, an outer layer and an inner mass. The latter had its origin from a delamination of the early cleavage cells or, in some cases, from an actual immigration of entire cells. From the inner cells the entoderm was formed, the outer row being the ectoderm. Metschnikoff (1886) confirmed the absence of invagination in *Lucernaria*, and also described the results of a study of *Nausithoë marginata* and *Pelagia noctiluca*. In the former, although the third and fourth cleavages were unequal, the

cells of the blastula were so nearly equivalent in size that no polar differentiation was possible. The blastula which (unlike *Aurelia aurita*) had a very large cavity, took on a somewhat elongate form, the cells of one side became thicker and at this point an invagination occurred. Even during the invagination the cells began to be differentiated from the ectoderm cells and when the blastopore had closed there was a typical planula with two layers differentiated. *Pelagia noctiluca* differed mainly in the wider extent of the invagination and in the absence of an early differentiation of the entoderm cells. Also during invagination the ectoderm grew so that the two layers came to be further apart than at first. The invagination in *Pelagia* was confirmed much later by Goette (1893). Metschnikoff thus confirmed the earlier workers on the formation of the entoderm by invagination, and he believed this process to be a wide-spread one in the Acraspedota [*Scyphomedusæ*].

This general belief in the wide extent of invagination among the *Scyphomedusæ* was vigorously attacked by Goette (1887), who claimed that *Aurelia* at least, and probably in other *Scyphomedusæ*, gastrulation had been erroneously described. This was due, in his opinion, partly to the fact that sections has not been used in the study of the gastrulation. Furthermore since a coeloblastula was the starting point of the gastrulation and a coelogastrula the end result, it seemed to be necessary and was natural to assume that in the stages between, an invagination had taken place. But "such an invagination does not occur in *Aurelia*" either as described by Haeckel or by Claus. A sterrogastrula is formed, " . . . and in *Aurelia* repeating exactly this process, the gastrulation occurs through a cell-inwandering." The cells which took part in the ingression came from the region of the blastula made up of shorter cells, and were either entire cells, or portions of cells set free by a process of delamination. The cleavage cavity thus became filled with a mass of cells, the entoderm, which by splitting apart assumed a position in a single layer. The ectoderm and entoderm then fused at one point and a prostoma was formed: "archenteron and prostoma therefore arise not through invagination, but through the hollowing out of a massive entoderm and

a breaking through of this cavity to the outside" (p. 5). Before the prostoma closes the embryo is exactly similar in appearance to an invaginate gastrula. Although he could not follow the entire process in *Cotylorhiza*, he found conditions which led him to believe that here also an ingression of cells was the process leading to gastrulation. This view was opposed to Claus and in 1891 this investigator gave the results of further observations upon *Cotylorhiza*, *Aurelia* and *Chrysaora*. In *Cotylorhiza* he found no ingression, but a true invagination. *Aurelia* showed some ingression of cells which he believed did not take part in entoderm formation; rather an almost typical invagination was the process as he had found earlier (1883). In *Chrysaora* he confirmed his earlier results.

As a result of his own work, and from a summary of that of others, Hamann (1890) held to Goette's views that polar ingression was the more common process of entoderm formation in Scyphomedusæ. His own observations upon *Aurelia aurita*, *Chrysaora* and *Cyanea capillata* led him to this conclusion, and from the papers of others he believed that a real invagination occurred only in *Nausithoë* and *Pelagia*, while at least ten other species of Scyphomedusæ showed a polar ingression. McMurrich's (1891) observations upon *Cyanea artica* seemed to confirm this, though he found the ingression to be multipolar. Although the end result was a structure like an invaginate gastrula, invagination had taken no part in the process, according to McMurrich.

Once more new evidence of invagination was brought forward by Smith (1891) working upon *Aurelia flavidula*. He found, indeed, that there was an ingression of cells, which might begin long before invagination started. This ingression resembled that found by Goette in *A. aurita*, but it was not of constant occurrence; indeed the majority of Smith's preparations did not show any ingression, though invagination occurred in all. He found further that only three or four cells took part in the ingression, the nuclei of these always broke up into small particles and later the cells degenerated as Claus also found. Sometimes they persisted till gastrulation was completed, but did not take part in the entoderm formation, some of them were even forced through the entoderm

into the coelenteron where degeneration occurred. Hence Smith concludes that ingression plays no part in the entoderm formation of *Aurelia flavidula*.

A comparative study of several species of *Aurelia* and of *Cyanea arctica* was made by Hyde (1894) who found that the cleavage might be regular or irregular, equal or unequal. The first and second cleavage furrows were meridional, the third equatorial. After the third division the cells were commonly smaller at one pole. In all species a blastula was formed, the cavity usually appearing first in eight or sixteen-cell stage. In *A. marginalis* a real delamination of the blastula cells occurred and was multipolar in extent, in this resembling *Lucernaria* (Kowalevsky), the inner cells formed an irregular layer and finally the prostoma broke through. In *A. flavidula* an ingression of entire cells, or a delamination, took place from different parts of the blastula wall and the cells thus free in the cavity assembled at one pole. This pole bent in to form a small funnel-shaped opening, the free cells grouped themselves about this in a layer, and the gastrula was formed. This is somewhat similar to the condition in *A. aurita* (Goette), the entoderm being formed from a small portion of the inbent wall of the blastula and from the cells which had migrated to this pole. A second method of gastrulation was found resembling that described by Claus (*A. aurita*) and Smith (*A. flavidula*), viz., an invagination, though a few cells might migrate from the wall and join with the invaginated cells. Hyde found that one of the characteristics of the cells which wandered into the cleavage cavity was that their nuclei were usually broken into small chromatin particles which were scattered through the cell, a condition noted earlier by Smith. In *Cyanea arctica* an invagination took place, but there were added to the invaginated cells some free cells arising by delamination from the blastula wall of the invagination pole. No migration of cells occurred in *Cyanea*. Thus in all three species Hyde finds a blastula with differentiated poles and in the cleavage cavity, often, a coagulated liquid and yolk grains. In the process of entoderm formation delamination occurred alone or in conjunction with other processes: in *A. marginalis* delamination alone; in *Cyanea* delamination limited to a



few cells at the pole where the invagination occurs, invagination being the chief process. In *A. flavidula* some blastula cells delaminate and some entire cells migrate into the cavity and these join a small invagination; or a large invagination occurs and this is only sometimes helped out by a few delaminated or migrated cells.

Hein's (1900) work upon *Aurelia aurita* gave results differing from those of Goette, Smith, Hyde, and others, in that he found it impossible to distinguish animal and vegetal poles because of the similarity of the cells of the blastula. He agrees with Claus and Smith that from different regions of the blastula wall a few cells may migrate inward, but these never take part in entoderm formation, degenerating sooner or later. He found also an occasional migration of entoderm cells into the coelenteron where they degenerated. An invagination, with a rapid division of the invaginated cells, led to the formation of the entoderm, the blastopore persisting as a very fine canal between the archenteron and the outside.

Goette (1900), in a short response to Hein's work, upheld the work of Hyde in all particulars, and though he acknowledged that invagination was the method of gastrulation in some cases, he still maintained the view that ingression plays a more prominent and active part among Scyphomedusæ. He discards entirely the view of Smith and Hein that the immigrated cells degenerate and take no part in entoderm formation.

C. W. Hargitt (1902a, 1902b) found that in *Cyanea arctica* the early cleavage stages were passed while within the gastric cavity or in the folds of the manubrium. Cleavage was total and regular, a typical blastula formed which by invagination gave rise to the gastrula.

Continuing his investigations upon Scyphomedusa, Hein in 1903 presented the results of work upon *Cotylorhiza tuberculata*. He found (as in *Aurelia aurita*) that a few cells migrated from the blastula wall into the blastocoel, but there they always degenerated. Gastrulation was by invagination, as he found in *Aurelia aurita*, but in *Cotylorhiza* the blastopore soon closed while in *Aurelia* it remained to form the prostoma. In neither species was



he able to find any proof for Goette's assumption that the immigrated cells took an active part in entoderm formation; hence he believes Goette to be incorrect in this point. Concerning these same cells Hein refers to the statements made by Hyde that the nuclei were usually broken into small fragments and scattered through the cell, and to the observations of Smith that no such cell ever had an intact nucleus. Hein concludes that such fragmented nuclei ". . . . legen wohl einen Zerfall der Zellen zum mindesten nahe."

More recently Conklin (1908) has studied *Linerges mercurius*. The eggs are deposited about 8 a.m. in masses surrounded by jelly. Polar bodies form typically, the sperm enters at the vegetative pole and moves to the animal pole where it fuses with the egg nucleus. Cleavage begins at the animal pole and at the end of the first cleavage there is a small space between the two cells which is the first indication of the cleavage cavity. The second cleavage is meridional, the third equatorial and both begin in the center of the egg and pass outward. The animal pole becomes the ectodermal pole, the vegetal the entodermal. Gastrulation is usually by invagination, though sometimes an ingression of cells from the vegetal pole fills the cleavage cavity, and the archenteron only later forms by a splitting apart of the cells. The end result is the same in the two cases, and this Conklin believes is an indication of the close relationship of unipolar ingression and invagination.

From this review it will be seen that the chief controversy with regard to the early development of the Scyphomedusæ has centered in the method of gastrulation. The differences in cleavage have been found rather insignificant and the results of gastrulation have in every case been the formation of a typical two layered planula. The main reason for the strict adherence to a belief in one or another single mode of gastrulation by some of the disputants appears to rest upon the belief that one mode must be more primitive than another; that there would be different phylogenetic relations indicated if this, rather than that, method were more common.

As early as 1881, Haeckel showed that there were marked varia-

tions in the gastrulation of *Aurelia aurita*; and the later work of Hyde upon three species of Scyphomedusæ showed other variations in the gastrulation even within a single species (*A. flavidula*). Hence there seems no reason further to insist upon the activity of any one, or any single process in entoderm formation. Nor does there appear to be any occasion to hold any longer to the view that differences in the gastrulation process have any necessary significance in phylogeny. Conklin has given expression to this view when he says (p. 163), " . . . the form of gastrulation is of no fundamental or general significance, but that it depends upon individual or environmental conditions."

The observations described in the following sections are not intended to add anything essentially new to the controversy regarding gastrulation, but to record the facts brought out in a study of *Cyanea arctica* and *Aurelia flavidula*, the development of the former species never having been fully worked out. It may be said, however, that the results of this study and a careful comparison of the earlier papers has led to the conclusion that invagination is probably a more general and dominant method of entoderm formation in the Scyphomedusæ than was thought by Goette, Hamann and others.

#### CYANEA ARCTICA

The development of *Cyanea* up to the formation of the planula takes place within the folds of the mouth lobes as stated in an earlier paper (1902 b). Cleavage stages are passed through rather rapidly so that only the later stages are found in most medusæ. Specimens obtained in early spring 1908, however, gave material which makes possible the study of the early development, though not the oögenesis and fertilization. In no case were there found in the mouth lobes more than an occasional egg which had not already begun to segment; hence the formation of polar bodies and fertilization must occur either within the gastric cavity, or before the eggs leave the ovary. The conditions in *Aurelia*, which are described later, suggest the probability that the formation of the polar bodies occurs at about the time of the escape of the eggs from

the ovary, and that fertilization takes place a little later in the gastric cavity.

The material obtained from *Cyanea* does not permit the determination of the cytologic details of the maturation process nor of fertilization. But it was found that two polar bodies were formed one of which sometimes divided again. The polar bodies are of considerable size, relatively larger than polar bodies in the *Hydro-medusæ*. The chromatin is usually enclosed within a membrane, but is collected into several more or less distinct masses, not forming a reticulum. The polar bodies remain attached to the eggs for varying periods, being occasionally found in blastulæ, though they commonly disappeared soon after the beginning of cleavage, probably through the rupture of the delicate egg membrane.

### *Cleavage*

The first division of the egg is meridional, the cleavage plane in some cases (figs. 1, 4) being almost co-incident with the plane of the polar axis, while in other cases (fig. 11) there was considerable divergence between these two planes. There result two cells which are nearly equal in size (figs. 1, 3, 4), though rarely exactly so, and in a good many cases there is a marked inequality (fig. 2) one blastomere being perhaps only about half the size of the other. This irregularity is also quite marked in the cleavage of *Aurelia*. It can be followed without difficulty in the living egg, and one finds upon looking over a series that a considerable number exhibit this feature, not only in the size of the blastomeres, but in their arrangement also.

When the first division is completed there is often present a small space between the blastomeres (fig. 12). This can be seen in entire eggs as well as in sections, and while not present in all eggs it persists, when present, to become a part of the large cleavage cavity. This early appearance of the cleavage cavity, not described by earlier workers, Conklin (1908) found to be also characteristic of *Linerges mercurius*.<sup>1</sup> He found among the organized sub-

<sup>1</sup>It may be doubted whether any such significance attaches to the cleavage cavity, as was earlier assumed, or whether the space referred to in the two cell stage can rightly be so regarded.—C. W. H.

stances an apparently more liquid substance, containing few yolk spherules, in the center of the egg. This he believes is the "precursor of the cleavage cavity." There was no evidence of such a substance in *Cyanea* eggs, but in *Aurelia* the center of the egg in a few cases showed what appeared to be a similar differentiation. It is also clear that in both *Cyanea* and *Aurelia* a liquid substance is present in the early cleavage cavity. It may be rather doubtful whether this comes from a substance already differentiated in the unfertilized egg, surely not in all cases.

In eggs of most animals the second division is meridonal and other workers have generally described the same thing in the Scyphomedusæ. So it was found in *Cyanea* (figs. 6, 11) that such might be the case. In view of the variations in the first cleavage it was not surprising to find instances (figs. 4, 13) where the second cleavage was equatorial, and this was not rare. This condition, of course, is often found in eggs which are erratic and irregular in cleavage as some of the Hydromedusæ, *Pennaria* in particular. It was possible to follow the cleavage in the living *Aurelia* egg and to observe that in some cases the second division was equatorial. When the first cleavage in *Cyanea* had been unequal a very common sequence was for the large blastomere to divide before the smaller giving a three-cell stage (fig. 5), a condition earlier noted in *Cotylorhiza* (Goette 1887) and *Aurelia* (Hyde 1894). The three cells were of approximately equal size and thereafter the cleavage was fairly regular.

After the second division, cleavage is more or less regular, but the cells are usually unequal in size. There appears to be no very definite sequence of division, so far as could be determined, nor was there any marked synchronism, since there were found 3, 5, 7, 8, 10, 12, etc., cell stages, as Hyde earlier noticed. The impression obtained from the study of many eggs was rather that of a certain independence of each blastomere in its division. In the 8-cell stage (figs. 7, 14) the cleavage cavity, which first appeared in the 2-cell stage (fig. 12) was larger, and this increase in size continued through the later stages until in the blastula there was a large cavity surrounded by a single layer of cells which were smaller at one pole (figs. 10, 16). The cells were often so crowded

together that some were pushed toward the outside, not reaching the cleavage cavity, and some toward the inside, not reaching the outer surface. This gave to the blastula something of the appearance of a double layer of cells. The cytoplasm was so filled with numerous closely packed yolk granules that cell boundaries were indistinct; the limits and number of cells must then often be estimated from the number of nuclei. As nearly as could be estimated about 400 cells are present in the blastula just before gastrulation. Figure 16 is a section of an early blastula with rather large cells; these by further division become much more numerous and smaller.

As pointed out by the senior author in a previous paper (1902 b, p. 555) gastrulation in *Cyanea* may be easily observed in the living egg to involve a very obvious invagination. This is just as evident in *Aurelia*. One has only to remove some of the eggs to a watch glass where the various phases may be followed from the beginning to the closure of the blastopore. The entire process is compassed in a comparatively brief time.

### *Gastrulation*

Sections of *Cyanea* show that the cells of the blastula are longer at one pole (figs. 15, 16); whether this is the animal or the vegetal pole could not be determined on account of the absence of the polar bodies at this stage. The first indication of gastrulation is the flattening of a portion of one side of the blastula, usually the thinner side as Hyde (1894) noted, followed by an invagination. Often at the first the mouth of the invagination is broad and open, but it soon narrows until the blastopore is almost closed (fig. 17). The closure may be completed before the gastrulation is finished (figs. 17, 18). When the invagination begins the coelenteron may be broad, though shallow; as the blastopore closes the coelenteron becomes smaller and may be almost entirely absent (fig. 18). Later this cavity again becomes more evident as is shown in the figure of the cross section (fig. 19), the invaginated cells more nearly filling the cleavage cavity. Still later these infolded cells, which are the primitive entoderm cells, come to lie closely against

the outer or ectoderm cells, entirely obliterating the cleavage cavity.

While more recent investigations have tended to discount the older views as to the importance attaching to the methods of formation of the primary germ layers, it will not be without some interest to briefly refer to some phases of the process. In addition to the invagination of cells there may be some immigration, though this is not common and may take place before or after invagination. Such cells appear to take no part in entoderm formation, and while their ultimate fate was not determined, they probably degenerate as was found by Claus (1891), Smith (1891) and Hein (1900, 1903). Some sections seemed to show that a delamination of the cells of the blastula might occur, the radially placed spindle of figure 15 clearly foreshadowing such a process. This is not at all a common condition and so far as could be determined the delaminated cells did not take part in the formation of the entoderm.

The nuclear spindles in cleavage stages are quite similar to regular spindles in other forms, having very clear, though delicate, spindle fibres with asters at either pole; in no case was it possible to demonstrate the presence of any centrosphere or centrosome. The chromosomes in cleavage spindles are well separated, but extremely small. In no instance was there found an amitotic division during the cleavage.

One of the most characteristic of nuclear phenomena in the cleavage stages of *Cynea* was found in the "resting" nucleus. In the majority of eggs, in early cleavage stages, the nuclei were composed of several vesicles (figs. 12-14, 20-24). Usually there were two of these vesicles almost identical in appearance and of equal size; other nuclei were single or composed of three to five vesicles, sometimes quite unequal in size (figs. 22, 24). These vesicles were found rather commonly in the cells of all eggs from the 2-cell stage to a blastula containing 24 to 48 cells, though not all the cells in any one egg contained the several vesicles. In the blastula just before invagination, and in the gastrula, they were less abundant and often entirely lacking, especially in the gastrula. Conklin (1908) in *Linerges*, sometimes found two equal chromo-

somal vesicles in the telophase of the first division and believes that they represent the gonomeres of Häcker (1902), *i. e.*, paternal and maternal nuclear constituents which have remained distinct and separate. Such a view is attractive, but the conditions in coelenterates other than *Linerges* seem not to bear out this interpretation. In *Cyanea* the presence of more than two vesicles, and of vesicles of unequal size, is rather opposed to this view. We are more strongly opposed to this suggested interpretation, because of facts presented in another paper (G. T. Hargitt, 1909) that some of the *Hydromedusæ* are even less regular and constant in this feature. For example in *Tubularia crocea* it was found that in the cleavage stages, up to what represents a blastula, such double nuclei, and indeed any chromosomal vesicles, were absent, while in the cells of the blastula and in the developing ectoderm and endoderm cells double nuclei were common. Thus in the earlier stages where the distinctness of sperm and egg chromosomes should have been more evident there was a total absence of this condition in *Tubularia*. As is well known also, Dublin (1905) found that nuclei in *Pedicellina* which appeared double in somatic and germ cells were not to be considered as representing gonomeres. These several points appear to offer an objection which it is difficult to overcome. Since in many animals there are often formed many chromosomal vesicles in the telophase of division and these later unite into a single vesicle, why may not these vesicles wherever found be better considered as simply stages in the reorganization of the nucleus, sometimes passed through and sometimes not, at least until it can be demonstrated in each case that the paternal and maternal nuclear constituents do actually remain distinct and separate. In *Cyanea*, *Aurelia* and certain *Hydromedusæ* at least there seems to be no question of the absence of any evidence of gonomery. Chromosomal vesicles often form, and may delay a long time before uniting into a single vesicle or may even fail to so unite—perhaps as a result of rapidly succeeding division—but there is no evidence in this to warrant the assumption of the distinctness of paternal and maternal constituents.



## AURELIA FLAVIDULA

*Oögenesis*

Sections of the ovary of breeding medusæ showed that young germ cells were still quite abundant, as well as quite a considerable number of older eggs, though the majority of the latter had been discharged. The eggs in the ovary secure their nourishment through the germinal epithelium to which they remain attached until mature; no absorption of other cells takes place.

In *Tubularia crocea* it was found (G. T. Hargitt, 1909) that the stages immediately following the last oögonial division were of great interest for it appeared probable that a synapsis occurred at that time. Attention given to similar stages in *Aurelia* gave the following results. The last oögonial division, which is mitotic, gave rise to small oöcytes in which the chromatin arrangement is not easily made out. There appears to be a spireme more or less massed together and concentrated toward one side of the nucleus and perhaps arranged in loops; no instance was found, however, in which this could be certainly determined. In older oöcytes (fig. 25) the chromatin was in a thread for only a short time, but all trace of a polar arrangement (if any existed) had been lost. The spireme disappears soon after growth begins and does not again reappear, in the eggs within the ovary, nor does a synapsis occur during the growth of the egg, for the stages of this period are sufficiently abundant to make it highly improbable that such a condition, if present, would be overlooked. One may only conclude then that if a synapsis occurs in *Aurelia* it probably takes place in the oöcyte just before growth begins.

The changes in the chromatin of the germinal vesicle during the growth period involve, (1) the disappearance of the spireme, already noted, and the formation of a reticulum; and (2) the condensation of the chromatin near the end of the period and the formation of the chromosomes. The reticulum in the early growth period (fig. 25) is wide meshed and the chromatin is mostly assembled into a few large, rather dense, flocculent masses. In older



eggs (fig. 26) the reticulum is more complex, fine-meshed and granular, and the chromatin is in a large number of granular, less dense masses. The diffusion of the chromatin does not go beyond this condition, a marked contrast to what happens in *Hydromedusæ*. In certain of the latter it has been found that characteristically the chromatin becomes so finely divided and so diffused that a stage is reached in which the nucleus appears to be without chromatin, though it is still present and later becomes more evident. While the reticulum in *Aurelia* will select an acid dye, the larger, denser masses always stain with the basic dye; hence the germinal vesicle never has quite the cytoplasm-like appearance that it does in nearly mature eggs of *Hydromedusæ*. Near the end of the growth period the granular masses of chromatin undergo a process of condensation, leading to the assumption of a more definite shape (fig. 27), straight or twisted strands, loops and rings, considerably smaller than the original chromatin masses. During the progress of these changes the nuclear membrane has become wrinkled, the egg has reached its maximum size, the nucleolus is degenerating; it is therefore clear that the time for the formation of polar bodies is near at hand and the chromatin changes described are the beginning of chromosome formation. In the eggs of many animals the chromatin rings and loops become chromosomes arranged in tetrads, in *Aurelia* these are apparently absent in the maturation spindles (fig. 28, 29).

### *Maturation*

The first stages in the formation of the maturation spindle were not found, unless figure 26 is one such stage. Here a single small aster centres in two granules (centrosomes) which have, perhaps, just divided. If this be the beginning of the spindle it is certainly precocious, for the egg is not full grown and the chromatin and nucleolus are in an earlier stage than in figure 27; but in the latter and other similar eggs there is no indication of asters or centrosomes. Whatever the genesis of the spindle, it is at first tangentially placed, as shown in the figure of the incomplete spindle, (fig. 28). Figure 29, a polar view of the first maturation

spindle, shows a later stage with all the chromosomes at the equator. The chromosomes are smaller and more numerous than in figure 28, giving evidence that a splitting has taken place. In fig. 28 there are about 9-10 chromosomes, in fig. 29, 18-20 and in cleavage cells at least 18-20, so it appears that before the maturation spindle has formed the conjugation of chromosomes has occurred. In the ovaries examined five eggs were found which showed the first maturation spindle forming, but none showed the second spindle and none the polar bodies. These facts, then, make it clear that the polar bodies begin to form just before or at about the time the eggs leave the ovary. In all probability this process is completed and fertilization takes place while the eggs are in the gastric cavity.

A comparison of figures 28 and 29 with figures 27 shows how small a portion of the germinal vesicle takes part in the formation of the chromosomes of the maturation spindle, a point already noted in Coelenterates (Conklin 1908, Smallwood 1909, G. T. Hargitt 1909). What has become of the rest of the nuclear contents? The chromatin in the maturation spindle (figs. 28, 29) is manifestly less than that in the germinal vesicle (figs. 25, 27), and no additional chromatin particles are found near the spindles. Many eggs show spherical bodies in the cytoplasm close to the nuclear membrane (fig. 25-27) sometimes with a vacuole about them, and the question arises: is this chromatin which has migrated from the nucleus before the rupture of the membrane? Such an interpretation has been given to apparently similar bodies in Hydromedusæ eggs by C. W. Hargitt (1904 a, 1904 b, 1906) and Smallwood (1909). These extra-nuclear bodies of Aurelia stain as intensely in the iron hematoxylin as do the chromosomes, but in hematoxylin-eosin they take the red or acid dye. This is evidence against their chromatic character, unless one assume that in the cytoplasm they change their staining reaction, a not improbable view. The conclusion must be reached that the greater part, at least, of the achromatic substance and superfluous chromatin at the time of the breaking of the membrane of the germinal vesicle escape into the cytoplasm and are rapidly absorbed or mixed with it. The relatively enormous size attained by the germinal vesicle

and the great amount of chromatin present, which does not take part in the formation of the definitive chromosomes, are points of considerable interest and significance, but are probably incapable of complete explanation in the present state of our knowledge. But may not a partial explanation possibly rest on the ground of a physiological need during the growth period, as earlier indicated by Wilson (1900, p. 128) when metabolism is extremely active, in the elaborating of reserve food material which shall furnish the energy for the following cell divisions and for growth? When the growth period is completed and the reserve food formed a considerable portion of the nuclear substance, now unnecessary, is cast aside, just how is of little importance.

The history of the nucleolus during the growth period contains some points of interest. This body in young oöcytes stains red in hematoxylin-eosin; in growing eggs sometimes red, sometimes blue or purple, even in eggs of the same size and of apparently similar age. Its composition is thus somewhat uncertain and its changes do not take place at any fixed or definite phase of the nuclear cycle. One or more small vacuoles are often present and each of these usually contains a small spot or granule which always stains red, regardless of the reaction of the rest of the nucleolus.

Near the end of the growth period the nucleolus becomes nearly transparent, but usually shows a deeply staining cap upon one side (fig. 27) which stains sometimes with acid sometimes with basic dyes. It appears as if substances were leaving the nucleolus during this period. Figure 27 suggests a connection between the nucleolar cap and the chromosomes as though nucleolar matter was passing into the chromosomes. This is not necessarily true, for the masses of chromatin are often present only in that side of the nucleus opposite the nucleolus and apparently with no connection between, though the reticulum undoubtedly furnishes an indirect pathway along which an interchange of material might take place.

A description of the cytoplasm at different stages will indicate what changes occur. In the young oöcyte (fig. 25) the cytoplasm

is composed of a dense mass of fine grains uniformly arranged, and staining blue (hematoxylin-eosin). The nearly mature eggs (figs. 27-29) have an alveolar cytoplasm, the walls of the large alveoli being made up of granules quite similar in size and staining reactions to the grains present in the young oöcytes. Within each alveolus is a more or less spherical body, not completely filling the vacuole, though of varying size, which always stains with the eosin. These are the yolk bodies and they are so abundant and so large that they give to the egg substance a red color with the stain used, the finer granules being hardly noticeable with low magnification.

### *Cleavage*

Although the cleavage of Aurelia was not followed in detail, it appears to differ little if any, from that of Cyanea, and a similar blastula is formed. Hyde (1894) found that the gastrulation process was somewhat different in material from Maine and in that from the Massachusetts coast. If the differences be correlated with the environmental conditions of the regions, our material (from Maine) should resemble that portion of Hyde's which came from a similar locality. As a matter of fact, however, the gastrulation proves to resemble that of her Massachusetts material, and confirms the results of Smith (1891) and our results on Cyanea, all from the more southern material.

The presence of cells free in the cleavage cavity was very rare and from many different stages of gastrulation it is evident that invagination is the chief, if not the only process. Fig. 30 shows an invagination just beginning, only a small portion of the blastula wall being involved. The invagination extends inward, assuming a condition similar to that shown in fig. 31; this extension of the infolded layer of cells being due in part to cell division and in part to a change in the shape of the cells. The closure of the blastopore, which soon takes place, begins inside and proceeds outward (fig. 33).

*Germ Layers*

One can find other sections of gastrulæ which appear to show an ingression of cells (fig. 32), but in all our preparations this is due only to orientation, for, if the gastrula be cut only slightly oblique, some cells of the entoderm are cut more or less transversely and give the impression of a mass of cells. Only when the plane of the section passes through the long axis of the invagination can the true condition of affairs be found. Whether the cells sometimes found free in the cleavage cavity do actually take part in entoderm formation may possibly be answered by the conditions show in fig. 34. Here is a condition similar, perhaps, to those found by Hyde where an immigrated cell was joining the invaginated cells. It is questionable, however, whether this cell is actually taking part in the entoderm formation for the invagination in this embryo is nearly completed and the entoderm cells arranged in a compact row except for the indentation caused by the cell 'b'. A similar cell, 'a', is present in the coelenteron; this together with the position of 'b,' and particularly the condition of the layer of entoderm cells near this cell, suggest the probability that cell 'b' is being forced, or is migrating, through the entoderm layer, into the coelenteron. Such cells in the coelenteron degenerate. Further evidence touching the fate of the cells free in the cleavage cavity is found in the condition of their nucleus. In agreement with Smith (1891), Hyde (1894), and Hein (1900) it was found that in *Aurelia* these cells rarely (in our preparations, as in Smith's, never) showed an intact nucleus, but there were only deeply staining granules present which may have been bits of a fragmented or degenerating nucleus. The presence of only scattered chromatin grains in the cells is pretty good evidence of the degeneration of those cells as Smith and Hein pointed out, though Goette (1900) and Hyde (1894) believe it does not warrant this interpretation. The entire absence of any sign of nucleus or even of chromatin fragments, which is true of some of these cells, suggests either a further degeneration of the cells or, more probably, that they are only cytoplasmic masses pinched off from some cell and hence doomed to degeneration. It is possible that in

some cases they only represent an aggregation of yolk granules and the coagulated liquid often found in the cleavage cavity, and are not cellular.

From the observations on *Cyanea* and *Aurelia* it is clear that at least in some cases there is no sign of an ingression of cells, or delamination, leading to entoderm formation, while in all cases invagination is the chief, if not the only process.

It was mentioned earlier, and is shown in figs. 33, 34, that the blastopore begins its closure inside and proceeds outward. The result is the complete separation of the entoderm layer before the lips of the blastopore have fused in the ectoderm. Since the gastrulation has been by invagination the primitive entoderm cells are already in a definite layer, and further changes in the two layers are only differentiations of the layers already present. The entoderm layer sometimes obliterates the cleavage cavity and lies closely against the ectoderm at a very early period even before gastrulation is completed (fig. 31). More often, however, the cleavage cavity is not filled till much later, and even after the embryo has begun to elongate into the planula there may be some space between the two layers (fig. 35, 36). When this elongation begins the cells of the ectoderm are long and slender and the nuclei are at the extreme outer ends (fig. 35), the cytoplasm being mostly limited to a very thin layer at the outer ends of the cells. The rest of the cell is packed with yolk bodies. There are some shorter cells which are limited to the deeper part of the layer. The entoderm cells are elongate, but relatively broad and rather few in number. Continued division results in the formation of long, very narrow, ectoderm cells (fig. 36) and, since the yolk is largely used up, the cytoplasm is more uniformly distributed and the nuclei are in the centre of the cells. The entoderm is still filled with yolk. The condition shown in this figure is one often met with and is evidence of the more rapid division of the ectoderm cells, thus pulling away from the entoderm layer. In some cases the two layers develop equally fast and remain closely joined at all times. From this condition, shown in fig. 36, the completed planula (fig. 37) is easily derived. The ectoderm uses up all its yolk, the cells, now completely and uniformly filled with cytoplasm,

have assumed their definite shape and relationship and the netting cells have formed from the more deeply lying ectoderm cells. The entoderm, continuing its differentiation, has come to lie closely against the ectoderm again. The cells do not become so long and narrow as the ectoderm and the cytoplasm is more vacuolated than in the ectoderm cells. The yolk is almost used up; the last portion remaining is limited to the entoderm cells of the anterior end of the planula. At the posterior end (lower in the figure) the ectoderm is already differentiating into what will become the point of attachment of the planula. The entire surface is covered with cilia, not represented in the figure.

The formation of the definitive ectoderm and entoderm is, then, only a differentiation in size, shape, etc., the position of the individual cells remaining relatively the same. There is never a solid mass of cells in the embryo such as one finds in the early planula of the Hydromedusæ, and the coelenteron is present from the first as the remains of the old archenteron of the gastrula.

Reference to the historical section will show that the difference in the results of this study and those of other investigators upon Scyphomedusæ are chiefly differences in detail. The irregularity and inequality of cleavage shown by Claus (1883), Haeckel (1881), Metschnikoff (1886), Hyde (1894) and others to be variable is here confirmed and shown to be even more variable. In some Hydromedusæ irregularity is the rule. However in these Scyphomedusæ the end result is the same whether irregularity or regularity is the chief character of the cleavage, and the variations and differences are then of only very minor importance. It has also been pointed out by many workers that considerable variation occurs in the process of gastrulation, but here again the end result is the same, viz., a typical planula, so that the differences are evidently of little significance. The methods of attaining the same result, though different, may only be indications of the individual differences of the eggs or of the environment as Conklin (1908) pointed out. The controversies over these unessential points only tend to show that attempts to bring these and other processes into exact and constant agreement with some assumed 'law' are futile, and often only indications of ignorance or bias.



## LATER DEVELOPMENT

*The Planula*

No attempt will be made to give technical details on phases of later development. There are yet problems involved in some of these which merit careful attention, and we much regret that our material does not include that necessary to such a study. It seems, however, that some brief review of a few points may be of value.

Referring to this phase Professor Agassiz has expressed views which seem more or less open to question. For example, concerning the time and circumstances associated with spawning he says, "it might be supposed that the great destruction of these animals by the autumnal gales would put an end to the development of the eggs of the stranded specimens, but this is not necessarily the case. On the contrary, I believe . . . that the coincidence of their spawning with the stormy season of the year is a provision to bring them into proper conditions for their future development and growth. Thrown among the rocks, upon the seaweeds, they become entangled and break up; but by the time they are in pieces the eggs, which have been accumulating in the little pouches formed by the folds of the margin of the arm, have reached their planula state, and are ready to swim about independent as animals as soon as they are cast off. . . . As with the returning tide such specimens are set afloat again, it is evident that their brood may frequently make its escape into the water and undergo their normal development after having been for a time ashore . . . . The young soon become attached to rocks, dead shells, or seaweeds, and assume their polyp-like condition. . . . The succession of fine days, along our shores during the month of October following the equinoctial gales, is the season during which the planulæ, set free by the decomposition of their parents, float about in search of a resting place." It is hardly necessary to point out the teleologic bias which vitiates this account. And it is only necessary to point out that while it might be plausible if only *Aurelia* were concerned, what shall be said of *Cyanea* or others whose spawning season is April instead of August?



In the earlier account by the senior author (op. cit.), it was shown that the planulæ might have variable life histories. It should be noted that these observations were made under the artificial conditions of the laboratory, and hence the variations might be due to the more or less artificial conditions. The same may also be said as to the scyphistoma.

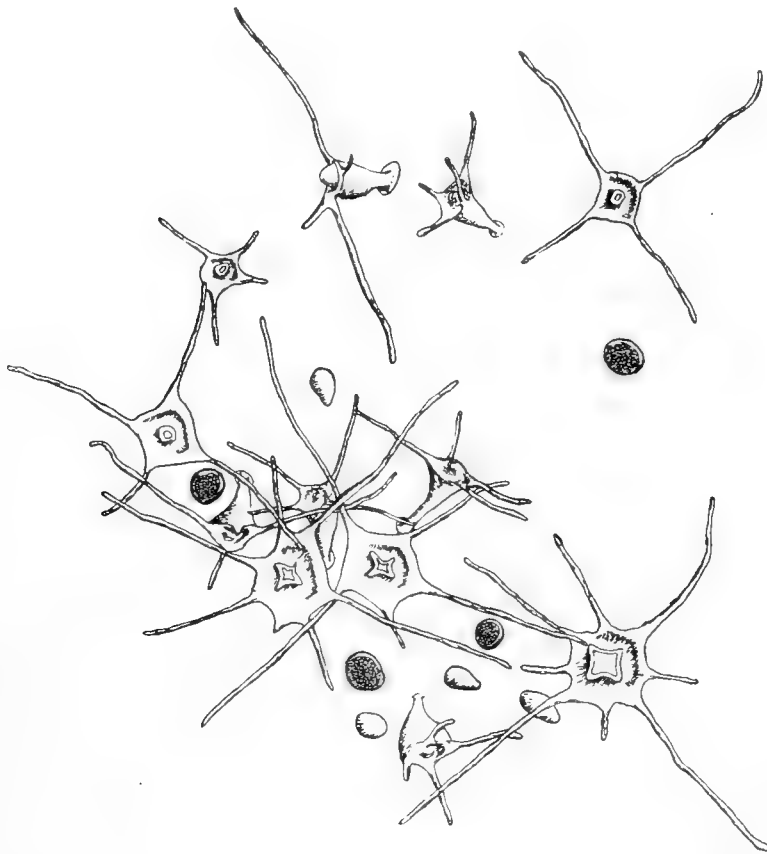
*Encystment.*—This is a condition often common where development is limited to the laboratory. Attention was called to this in the earlier account. Figures 38–40 show the aspect of young polyps just emerging from the cysts, which in these cases become floats, by means of which the polyps may be borne for some time. Whether such a condition ever occurs in nature we have no means of knowing, but so far as recalled it has not been made a matter of record. All the observations point to the conclusion that the phenomena associated with encystment are expressions of adaptation due to unfavorable conditions of environment; and this may serve to reconcile certain more or less conflicting accounts of earlier observers, more especially those of McMurrich (1891), and Hyde (1894).

### *The Scyphistoma*

Concerning this phase the earlier work of L. Agassiz was perhaps the best of his entire account. Many of his admirable figs. (cf. op. cit. pl. xi), of both *Aurelia* and *Cyanea* would illustrate our own results quite fully.

The account here given relates almost exclusively to *Cyanea*, though scyphistomæ of *Aurelia* have been kept under observation at several times during the progress of these studies. As in the life history of the planula, so in that of the polyp there is much variability. In a small proportion of specimens there was a metamorphosis into the ephyra within a period of about twenty days after the planula attached itself. In the larger number the period was much greater than this, thirty to forty days under average aquarium conditions, while in some cases there was no transformation even at the end of two months. In the case of *Aurelia* the polyp life is apparently much greater, usually several

months or throughout the entire winter season. The senior author has taken polyps of *Aurelia* undergoing strobilation and giving birth to ephyræ during the month of April in a locality where during the former summer he had kept some of the same brood of polyps under constant observation for a period of more than a month without finding any sign of metamorphosis. This



Sketch (camera) of colony in watch-glass, showing various phases from planulæ to scyphistoma.

is in confirmation of the observations of Agassiz (op. cit. p. 77), who reports strobilation as occurring in the month of February and March.

The polyps feed readily upon various minute organisms, such as larvæ of echinoderms, copepods, etc., and in the aquarium have been found to turn cannibal and devour planulæ, instances of which we have found several times, in a few cases having wit-

nessed the entire operation. As compared with *Aurelia* the polyps of *Cyanea* are relatively small; they are whitish in color, making a most beautiful sight when viewed together in a watch glass by reflected light against a black background. (cf. text fig. 1.).

*Stolonization*.—This is much less common than in *Aurelia*, though not rare. In fig. 42 is shown such a case. Stolons may arise from the body or base of the polyp as shown in the figure, and may be very delicate thread-like structures, or often somewhat massive, and these may sub-branch, thus giving rise to little colonies of polyps formed by this mode. The budding of polyps directly from the body of a parent was not observed in *Cyanea*, but is not uncommon in *Aurelia*.

*Strobilation*.—This feature is rather inconspicuous in *Cyanea*, owing to the relatively small size of the polyp, and the strobilæ are relatively few in number, often but a single one arising from a given polyp at a given time. In other cases the polyp becomes polystrobilous, from three to five ephyrae being set free in early succession. In figs. 44–46 are shown phases of strobilation as drawn from nature by Mr. H. B. Bigelow, for which kindness it is a pleasure to express thanks.

It remains to mention another feature, namely, that concerning the origin and development of the tentacles. These arise by a process of budding from the margin of the peristome, and are usually four in number, constituting the primary set. In many cases, however, only two tentacles appeared at first and on opposite sides of the mouth; later two others would arise in the appropriate intermediate positions. In a few cases the polyp seemed organized in a trimerous fashion, three primary tentacles arising about a triangular mouth, to be followed later by a second set of three tentacles at intermediate positions, rendering the specimen hexamerous. The average number of tentacles in the polyp of *Cyanea* is sixteen, though this number is not definite, as many were to be found having twenty or more. In a few instances bifurcated tentacles were found, a not unusual condition among coelenterates.

*The Ephyra*

Touching this phase occasion will be taken to mention chiefly one feature, that relating to metamorphosis from the strobila. One of the first general signs of this change is that of the apparent atrophy of the scyphistomal tentacles. Concerning this feature Agassiz (op. cit.), says "at this time the wreath of tentacles which crowns these bodies is cast off, and during the fair days of that season, in the month of March or early April, the saucer-like disks of the strobila begin to separate." As a matter of fact the tentacles are not "cast off" at all, but are resorbed, as is well known. Various accounts have been given as to just how this takes place. According to Bigelow (1900), it is by a process of degeneration. According to Friedemann (1902), it is by a complication of modes, namely, a strangulation of the base of the tentacle, partly through the crumpling and atrophy of the tissues, with the coöperation of the phagocyte cells. "Die Rückbildung der Tentakeln erfolgt theils nach vorhergehender Einschnürung an der Basis und nachfolgendem Abwerfen, theils durch Schrumpfung und Atrophie des Gewebes mit Hilfe von Phagocytären Zellen."

These accounts do not seem to be confirmed, except in a limited degree, in the case of *Cyanea*. There is not apparent at any time the degenerative aspects described by Bigelow. Nor has the basal constriction referred to by Friedemann been observed. That something of phagocytosis may be involved seems altogether probable, though definite evidence of the actual operation of such phagocytic cells has not been observed. In one case actually followed from beginning to end it was found that some twenty-four hours were involved in the process. As is therefore evident, it proceeds slowly, a fact further suggesting the operation of resorption, due in part perhaps to phagocytosis, and perhaps in part to the direct influence of contiguous tissues. The latter would seem to be the more important and active of the two. That there is no marked evidence of distinctively degenerative processes involved may be inferred from the fact that for some time after resorption is under way, indeed till far advanced, the tent-

acles retain their irritability and active response, contracting after stimulus quite as usual, though less vigorously or promptly. This is more evident as resorption approached completion. This would seem to be what might naturally be expected. It seems, therefore, that it may be said that the reduction or atrophy of the tentacles is due primarily to direct resorption unaccompanied by distinct evidences of degenerative changes. There was evidence to suggest that in the process of tentacle resorption, those of the primary series, *i.e.*, the perradial and interrarial, were first involved, followed by those of the later series, but at irregular intervals. However, there was much variation in this respect, each tentacle behaving more or less in an independent or individual manner.

Except for certain theoretical considerations we might dismiss the subject at this point. As is well known, it has long been a rather current and general assumption that in Scyphozoa the rhopalia, or so-called sense organs, are metamorphosed tentacles. So wide spread is this view that one can hardly consult any of the current text-books of zoölogy in which it is not asserted without qualification or doubt. As an example the following citation from Hertwig's *Lehrbuch*, (Kingsley's translation, p. 246), may be given as a fair illustration. "Instead of a nerve ring there are eight nerve centers connected with the sensory pedicels. Each of these pedicels is a modified tentacle with an entodermal axis and an outer layer of ectoderm."

In connection with experiments on regeneration in Scyphozoa (1904), the senior author had occasion to express serious doubts as to the validity of this assumption. Later study and research has tended to confirm the doubt, and has strongly impelled the conclusion that there is no genetic relation whatsoever between these organs. Critical study of the actual process of tentacle resorption shows it to be purely physiological, quite as much so as that of such processes generally. It would be the height of absurdity to suggest that the urostyle of the frog's skeleton might be a metamorphosed polywog tail; but hardly more so than the one here under review!

But aside from physiological objections, abundant facts of morphology go to discredit the assumption. Not to suggest such a priori reasons as that out of the large number of scyphistomal tentacles only eight, or in some cases four, should be thus modified, it may be worth while to call attention to the more significant fact that medusæ are well known whose development is direct, and consequently have no scyphistomal tentacles from which rhopalia might be developed. Nor does it suffice to say that in such cases heredity may have established the condition. But other facts are significant. In the case of polydisc strobila only the primary ephyra as a rule has had the requisite tentacle primordium for such transformation; others of the series should be found to lack sensory bodies, if the assumption be valid. But of course this is not the case. Furthermore, as already intimated, during the experiments on regeneration referred to there was not the slightest evidence that any tentacular primordium was necessary, or in the slightest degree concerned in the process. As is well known, it is not rare in such experiments to find appearing some heteromorphic organ arising in the course, such as the occurrence of a tentacle instead of an eye (crustacea). Not the slightest evidence of the sort was found in the case under consideration. As may be noted (*op. cit.*), regeneration proceeded directly from the entoderm of the marginal pockets, or rarely from other portions of the margin. It was also practicable to trace step by step the histogeny of the organs from start to finish.

This view is supported also by other observers. Bigelow (1900), pointed out that in *Cassiopea* the rhopalia arose prior to the atrophy of the tentacles; and Friedemann (1902), p. 264, says explicitly that the sense bodies are not transformed tentacles. These observations go to confirm the earlier view of Goette (1887), that the sense organ can not be considered as homologous with the tentacle of the scyphistoma.

We seem to have here another illustration of the baleful consequences of uncritical subservience to theory. It may be doubted if in the original hypothesis any attempt of a critical character was made to work out the primary genesis of the organs in question. To the time of the above mentioned experiments the writer has

to confess that it had not occurred to him to question the validity of the assumption, and in the present case submits such facts and inferences as have seemed to bear more or less directly upon the problem, and seem to warrant the strictures expressed.

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## EXPLANATION OF FIGURES.

Drawings of figures 1-37 have been made with the aid of the Abbé camera lucida. The magnification indicated in each case is the original magnification; the figures have been reduced to  $\frac{2}{3}$  the original size.

FIGS. 1-10. *Cyanea arctica*, drawings of entire eggs.  $\times 360$ .

FIG. 1. Two-cell stage, showing polar bodies. Cells slightly unequal in size.

FIG. 2. Two-cell stage, cells unequal.

FIG. 3. Two-cell stage, cells equal.

FIG. 4. Two-cell stage, with polar bodies; the nuclei have divided the second time and the ensuing division would have been equatorial.

FIG. 5. Three-cell stage.

FIG. 6. Four-cell stage, polar view.

FIG. 7. Eight-cell stage, showing cleavage cavity.

FIG. 8. Sixteen-cell stage, all cells outlined.

FIG. 9. Twenty-four-cell stage.

FIG. 10. Blastula near the end of cleavage, at least 200 cells present.

FIGS. 11-19. *Cyanea arctica*, from sections.  $\times 715$ .

FIG. 11. Two-cell stage, showing polar bodies; the spindle shows that the second division would have been meridional.

FIG. 12. Two-cell stage with 'resting' nucleus multi-vesicular. The beginning of the cleavage cavity is shown.

FIG. 13. Four-cell stage. Three of the cells only are shown and multi-vesicular 'resting' nuclei. Polar bodies present, the second division has been equatorial.

FIG. 14. Eight-cell stage, with cleavage cavity and multi-vesicular nuclei.

FIG. 15. A blastula of 24 cells.

FIG. 16. An older blastula of about 300-400 cells.

FIGS. 17-18. Longitudinal sections of young gastrulæ.

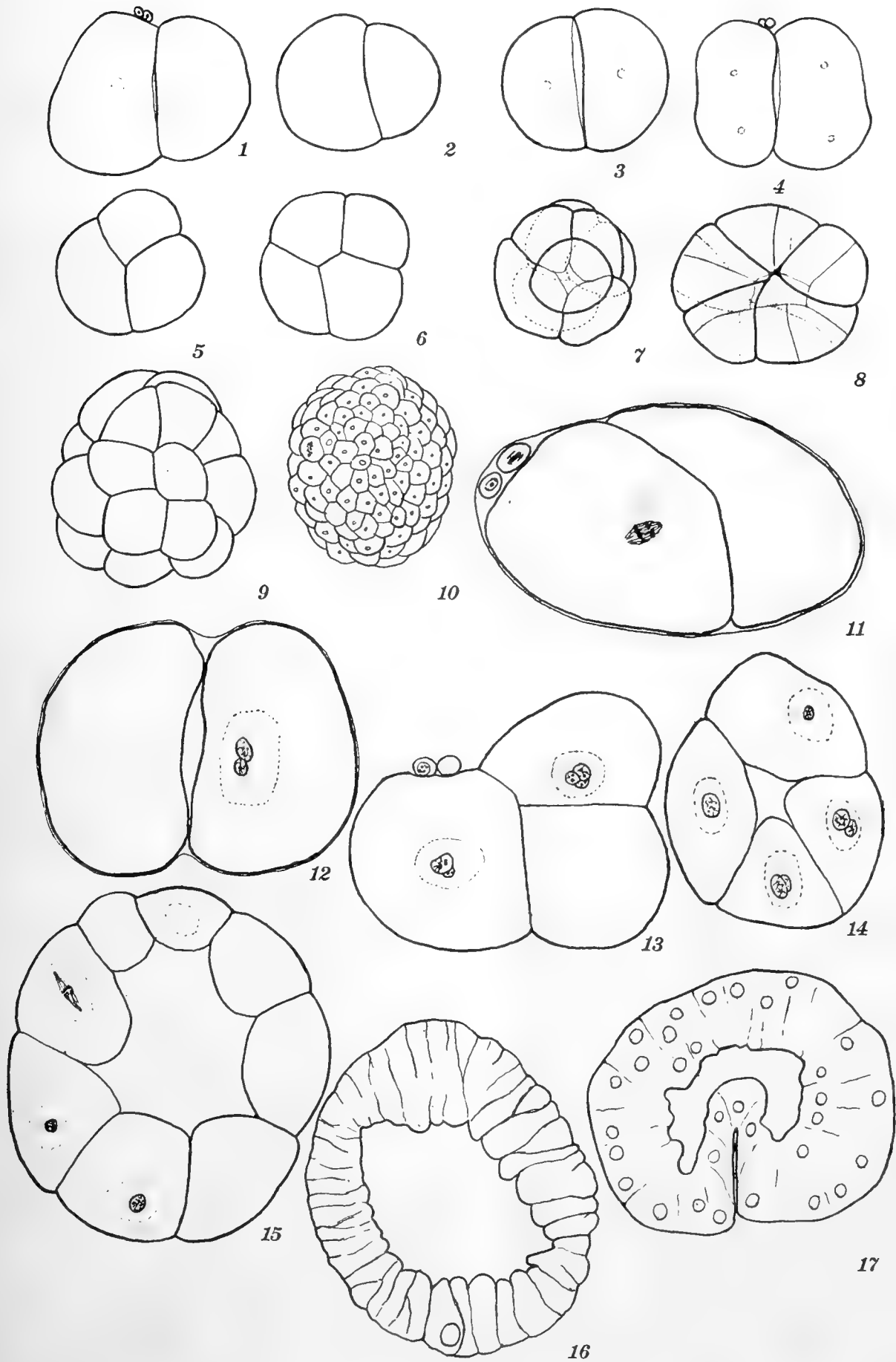


FIG. 19. Cross section of an older gastrula showing the cleavage cavity and coelenteron.

FIG. 20. *Cyanea arctica*. Unequal double nucleus from 4-cell stage.  $\times 1340$ .

FIG. 21. *Cyanea arctica*. Bilobed nucleus with vesicles equal, from 2-cell stage.  $\times 1340$ .

FIGS. 22-24. *Cyanea arctica*. Multi-vesicular 'resting' nuclei from cleavage stages.  $\times 1600$ .

FIGS. 25-29. *Aurelia flavidula*. Sections from ovarian eggs.  $\times 1900$ .

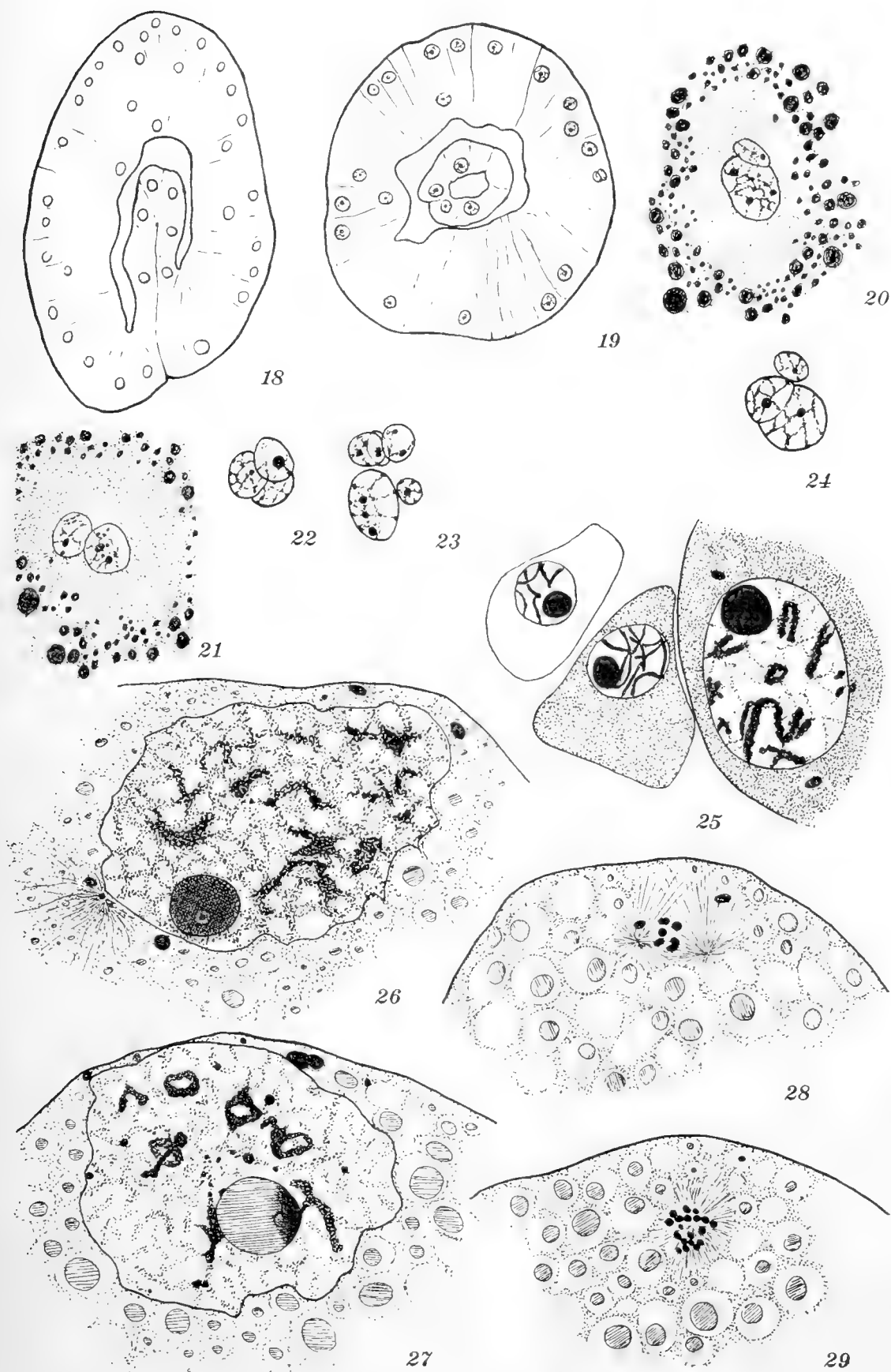
FIG. 25. Germinal vesicle from an egg about  $\frac{1}{3}$  grown. Two small oöcytes also shown, at beginning of growth; chromatin in a spireme.

FIG. 26. Egg approaching maturity, though not fully grown. An aster and two centrosomes present. Chromatin in masses scattered through the reticulum.

FIG. 27. End of growth, the chromatin condensing to form chromosomes.

FIG. 28. First maturation spindle forming, chromosomes not all in the spindle (some chromosomes in another section).

FIG. 29. Polar view of completed first maturation spindle which is still tangential. A splitting of chromosomes appears to have taken place. (All chromosomes present in this section).



FIGS. 30-37. *Aurelia flavidula*. Sections of developing eggs.  $\times 715$ .

FIG. 30. Very early stage of gastrulation. Yolk bodies present in all cells. In the cleavage cavity is a coagulated liquid.

FIG. 31. The invagination is nearly completed. The cells of the invagination are few in number and nearly cubical; some of these cells dividing.

FIG. 32. An oblique section giving the appearance of a cell ingression. This is only apparent, for such an ingression does not occur.

FIG. 33. Invagination completed and the blastopore closing, the entoderm layer already being separated.

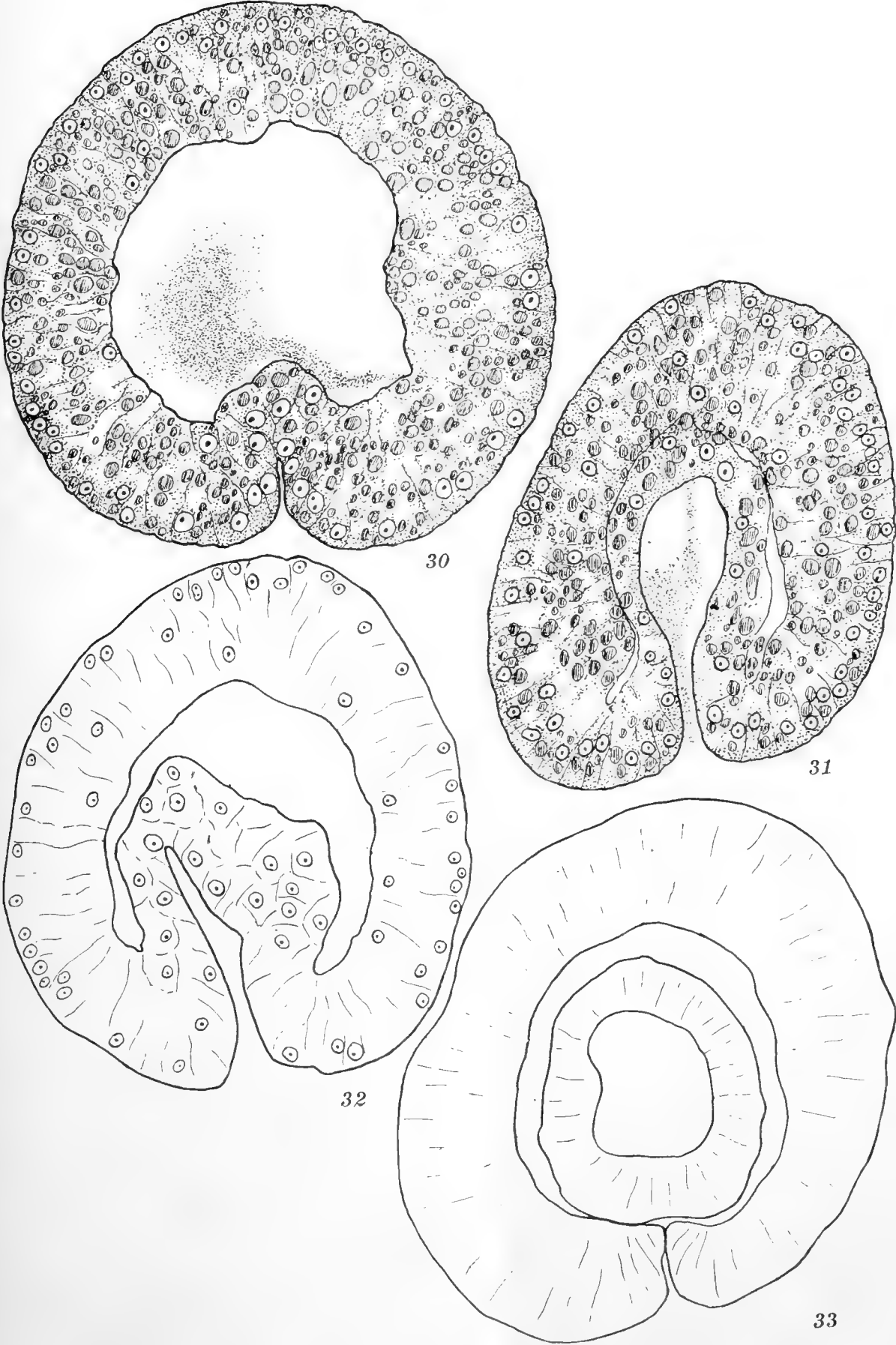
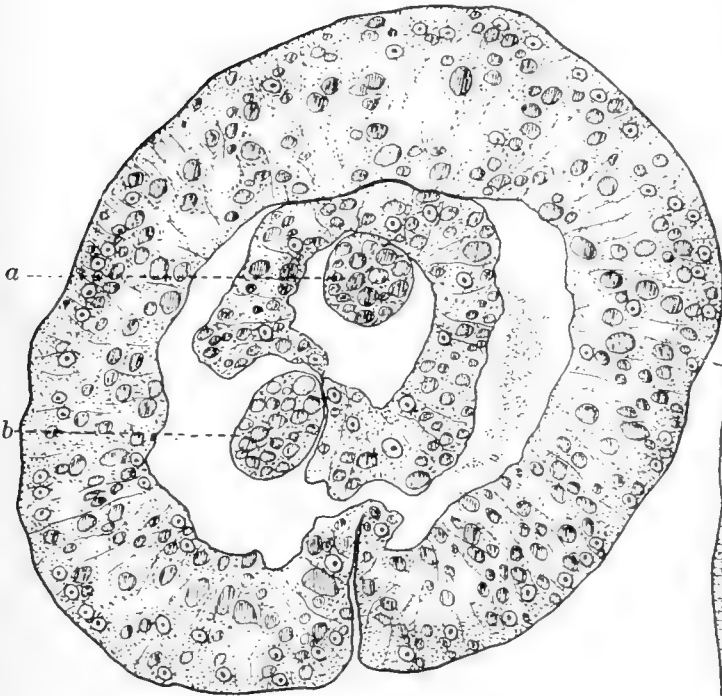


FIG. 34. Invagination completed, the entoderm completed and the blastopore closing in the ectoderm layer. 'a' a cell in the coelenteron, 'b' a cell in the cleavage cavity which is apparently migrating into the coelenteron. Drawing made from 2 sections.

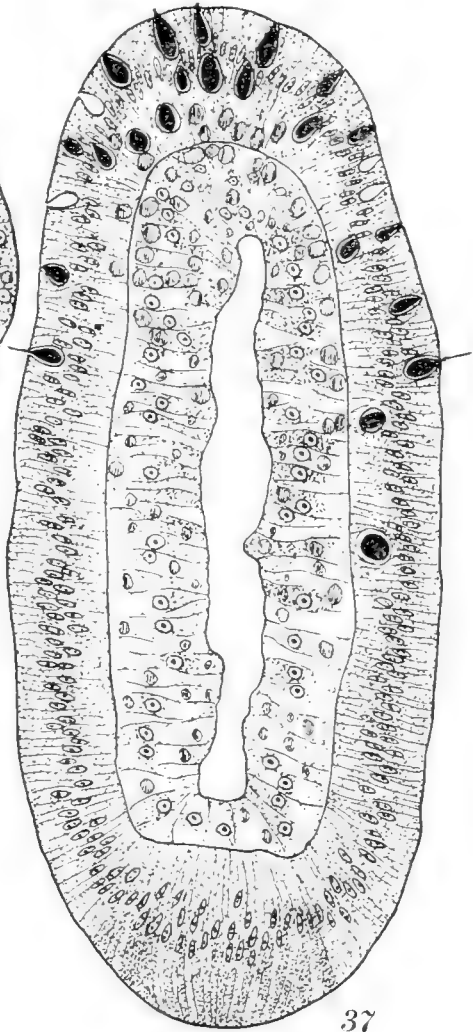
FIG. 35. Cross section of a very early planula, showing coelenteron and cleavage cavity.

FIG. 36. Longitudinal section of a developing planula. The ectoderm cells have nearly their definite shape and size and in their rapid growth have pulled away from the more slowly growing entoderm.

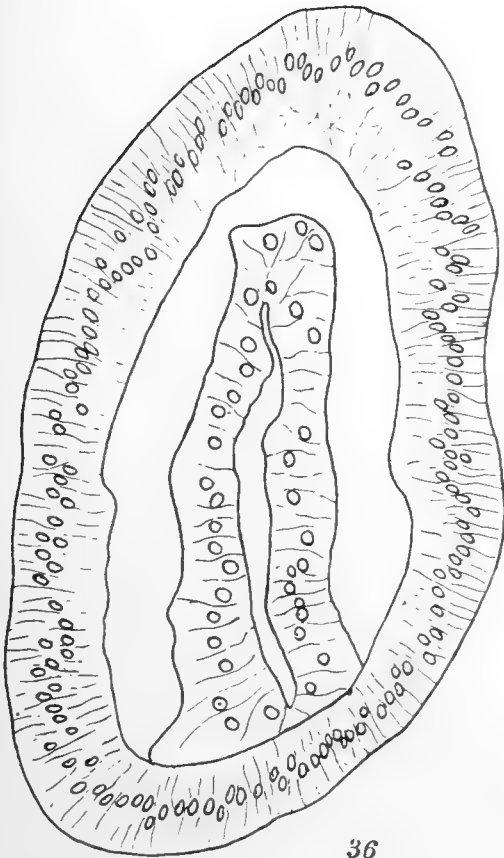
FIG. 37. Completed planula, anterior end uppermost. The differences between the ectoderm and entoderm are well shown. The yolk is used up except for a few granules at anterior end. The black bodies are nematocysts. At posterior end the entoderm is differentiating to form a point of attachment for the planula.



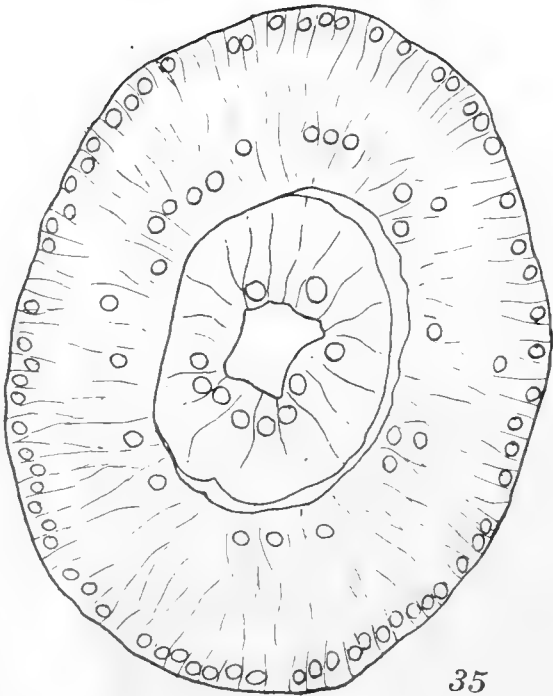
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37



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FIG. 38. Figures *a* and *b* show encysted condition, and polyp just emerging from cyst, which serves as a float.

FIGS. 39-40. Floating polyps at slightly varying stages of growth, and still floating on portion of cyst.

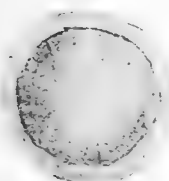
FIG. 41. Young polyp just emerged from cyst.

FIG. 42. Stolonization, a young budded polyp at left.

FIG. 43. Scyphistoma about at full development.

FIGS. 44-46. Phases of strobilation, in the last the disks quite deeply divided.

FIGS. 47-48. Late phases of metamorphism, in the last the young ephyra ready to be liberated.



a



39



40



41

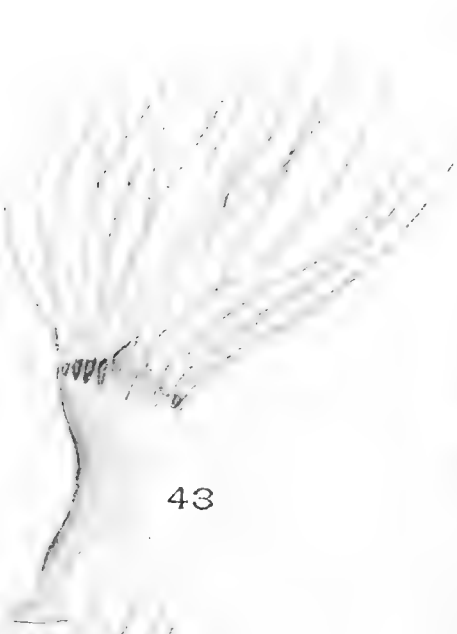


b

38



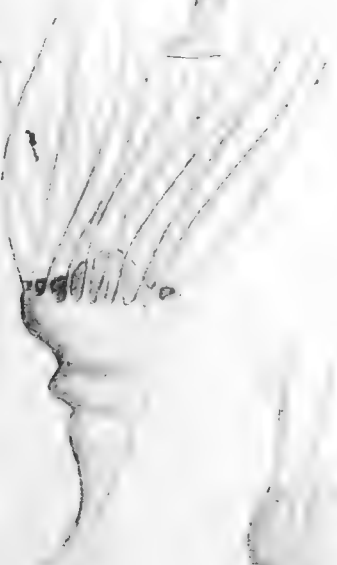
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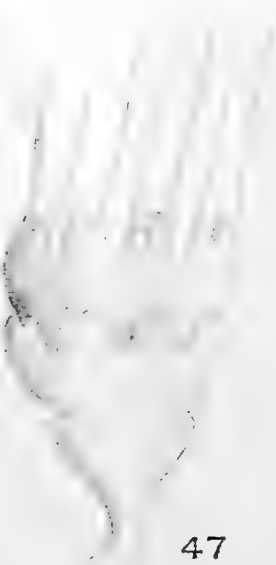
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# THE HISTOGENESIS OF THE BLOOD PLATELETS

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WITH TWO COLORED DOUBLE PLATES

The theories that have been proposed regarding the nature and origin of the blood platelets, before the publication of my papers on this subject in 1906, may be grouped and briefly discussed under the following headings.

1. *The blood platelets are fragments of the leukocytes.*

The principal objection to this view is that the blood platelets when observed and stained in various well-known ways present such a characteristic structure and appearances as to make such an origin very improbable if not inconceivable.

2. *The blood platelets are derived from the extruded nuclei of the red blood corpuscles.*

The principal objection to this view is that the extruded and degenerated nuclei of the red blood corpuscles never show any similarity in appearance to, nor any transitions toward, blood platelets in preparations in which both of these elements are present and characteristically stained. I have been able to follow all stages in the fate of these extruded nuclei up to their final dissolving in the plasma in preparations of embryonic blood and of the blood of animals which had been intravenously injected with the hemolytic toxins, ricin and saponin. In these preparations in which the extruded nuclei of the red blood corpuscles are abundant I have in no instance seen a suggestion of the formation of a characteristic blood platelet from the extruded nucleus.

3. *The blood platelets are derived from certain parts or constituents of the red blood cell other than the extruded nucleus.*

Various theories are grouped under this heading. The fundamental objection to all of them is that the most approved methods of preparation fail to give satisfactory evidence that the red blood cell at any period in its development contains a body which suggests a blood platelet and do not show any transitions between red blood corpuscles or their fragments and the blood platelets. Occasionally in smear preparations a blood platelet may be seen lying upon a red cell and this appearance has been interpreted as showing that the blood platelet is first contained within a red blood corpuscle, then later is extruded. In sections, in which the blood platelets are characteristically stained, I have never seen a blood platelet within a red blood corpuscle. The view that some, if not all of the blood platelets, are pinched-off portions or fragments of the red cells which have undergone certain changes whereby they present the characteristic staining and structure, which are brought out by the modern methods of preparation, is a pure assumption of fact to explain the obvious differences between fragments of red blood corpuscles and blood platelets. Fragments of red blood corpuscles, or so-called blood platelets containing hemoglobin, practically do not occur in the normal blood.

4. *The blood platelets are a definite and independent kind of blood cell.*

This is open to the objection that the blood platelet has no nucleus. The central granular portion brought out by proper staining methods cannot be regarded as a nucleus as has been maintained because it lacks the definite structure of a nucleus and the characteristic affinity for nuclear dyes.

5. *The blood platelets are not independent cells or cell fragments but are of the nature of albuminous precipitates.*

Against this view may be urged the characteristic appearances and structure of the blood platelets as brought out by various methods of preparation and the fact, confirmed by personal observation, that the blood platelets under certain conditions show continuous and amoeba-like change in form and outline.

6. *The blood platelets are cells out of which the red blood corpuscles develop.*

That they have nothing to do with the development of red blood corpuscles is shown by the absence of transitions between them and young red blood corpuscles in preparations from bone marrow appropriately stained, and also by well established facts in the development of the red blood corpuscles.

In brief, after much study of the subject and for the reasons given, as well as for many others, I am compelled to believe that all these theories of the origin and nature of the blood platelets are erroneous and untenable.

As one of the results of an extensive study of the blood and blood forming organs of various animals I have become convinced that the blood platelets are detached portions of the cytoplasm of those giant cells of the blood forming organs which have been named megakaryocytes by W. H. Howell to distinguish them from the multinucleated giant cells of the bone marrow, the so-called osteoclasts or polykaryocytes.

This view of the origin and nature of the blood platelets is based upon the following:—

#### FACTS AND OBSERVATIONS

By means of a special staining method, devised by me, which gives the so-called Romanowsky polychrome staining, I have been enabled to stain characteristically the blood platelets in sections of fixed tissues and organs so that they may be positively recognized and may be clearly distinguished from other histological elements.

The description of this method of staining will be found at the end of this paper.

I have studied especially sections of the bone marrow and spleen of the cat and young kitten. Similar material from man, mouse, dog, rabbit, guinea pig, white rat, and opossum has also been studied. The appearances observed in the preparations from the blood forming organs of man and of these animals indicate that the blood platelets originate from the megakaryocytes in all of them.

In sections of the blood forming organs, and of blood vessels containing blood, stained by this special method, the blood platelets present the following appearances and characteristics. They consist of a hyaline blue staining substance in which are imbedded closely set, minute, red to purple staining granules. In deeply stained preparations this hyaline ground substance may not be apparent. Their well known disc shape is sometimes preserved so that they may appear as short rod-like bodies when viewed in certain positions. Their margins may be smooth or show irregular, small projections, of the hyaline ground substance. Elongated forms, sometimes several times as long as broad, occasionally occur. These have been described by F. Weidenreich in smear preparations. The red to purple staining granules may be aggregated in a more or less sharply outlined mass in the central part of the platelet so as to suggest a nucleus surrounded by a hyaline cytoplasm. In some platelets a clear, unstained, more or less sharply outlined vacuole-like area may be seen in the midst of the granules. This has also been noted in smear preparations by G. Schmauch and by F. Weidenreich.

The structure of the blood platelets, and especially the peculiar color taken by the granules within them, are very characteristic and sharply distinguish them from the other elements of the blood. The red corpuscles and granular precipitates caused by certain fixing agents in the plasma stain pink or green depending on the fixative used and the intensity of the staining. The nuclei of the leukocytes stain blue and the various kinds of granules in the cytoplasm of the leukocytes, according to the species of the animal, may or may not be characteristically stained as in smear preparations stained by modern blood staining methods.

Blood platelets, with the staining and other appearances above described, are found in sections of vessels doubly ligated during life in numbers corresponding to the numbers of the blood platelets in fresh blood. Moreover, in sections of material properly fixed by formaldehyde, in which no granular precipitate is produced, all bodies which are of the shape and size of blood platelets stain characteristically. For these reasons it is believed that all of

the blood platelets are stained by this method and that the blood platelets are all of the same nature or kind.

Fixation in methyl alcohol or in corrosive sublimate produces in the plasma a more or less abundant precipitate in the form of granules of a size and shape closely resembling the blood platelets and which cannot be distinguished from blood platelets in preparations stained by the usual methods. This granular precipitate has doubtless been confounded with blood platelets by observers who have found an apparent increase of blood platelets in doubly-ligated vessels and in stagnant blood, or apparent blood platelet thrombus formation after cauterization of the vessel wall, because they did not use a specific staining method for the blood platelets in their experiments.

In sections of the blood forming organs the blood platelets may be very small in number because the blood has flowed out of them, taking with it the blood platelets, and because, with the stopping of the circulation, the platelets become irregularly distributed throughout the vessels. They are very numerous in the spleen, which is readily accounted for by the consideration that the structure of that organ favors the accumulation of various kinds of cells and blood corpuscles within it and prevents their escape from small pieces cut from it.

The giant cells or megakaryocytes in sections of the blood-forming organs present the following peculiarities which are of importance for the subject of this paper.

In the cytoplasm of the megakaryocytes or the giant cells are imbedded more or less numerous red to purple staining granules identical in appearance and staining with the granules in the blood platelets. Also in the cytoplasm small vacuole-like unstained areas may be seen like those in the blood platelets. In preparation fixed by methyl alcohol the granules may be arranged in more or less definite parallel rows or lines coursing in various directions.

The cytoplasm of a minority of the megakaryocytes is prolonged into pseudopod-like processes of varying size, shape and number. These processes, which will hereafter be referred to as pseudopods, often occur as bud-like projections of the size of a blood platelet or as strands, of a width corresponding to that of a platelet, which



may attain a length greater than that of the diameter of an oil immersion field. Nearly all of the cytoplasm of some cells may be formed into pseudopods projecting in various directions.

The pseudopods commonly are seen to project into more or less well defined blood channels. This is especially clearly shown in the spleen of the young kitten where larger and smaller pseudopods are frequently seen projecting into veins through small openings in the vessel wall. A whole megakaryocyte in active pseudopod formation has been found in a blood vessel in the spleen.

The amount of cytoplasm associated with a megakaryocyte nucleus varies and such nuclei showing the usual signs of degeneration and senility with little or no cytoplasm about them are frequent. This is due to the well known fact that the megakaryocytes lose their cytoplasm. Thus pseudopods not connected with megakaryocytes occur not rarely.

The characteristic granules are most numerous and most closely crowded together in the cytoplasm of the larger megakaryocytes, of those with pseudopods, and of those in process of losing their cytoplasm and in detached pseudopods. The marginal or peripheral portion of the cell body is usually free from granules, is hyaline, stained blue and is sharply demarcated from the granule-containing cytoplasm. The border of the cell may be smooth or show rounded or irregular projections of the hyaline cytoplasm. In the pseudopods the granules may be abundant throughout the cytoplasm or they may occupy only the axial or mesial portions leaving a sharply demarcated narrow marginal zone of hyaline blue staining cytoplasm, the borders of which may be smooth or show small projections of varying shape, just as does the hyaline blue stained marginal portion of the blood platelets. Thus some of the smaller pseudopods are identical in appearance with the elongated forms of the blood platelets in everything except that they are in continuity with the cytoplasm of the megakaryocyte.

In some megakaryocytes or pseudopods one or more small groups of the granules may be seen more or less definitely separated by a narrow zone of hyaline cytoplasm from the rest and arranged in one or more round or oval, more or less sharply outlined masses which are identical in appearance and staining reaction with the

masses of granules in the blood platelets and may also contain vacuole-like unstained areas like those in the blood platelets. Such a small mass of granules is commonly found in a small bud-like pseudopod and the appearance is thus produced of a platelet, the hyaline ground substance of which is continuous with the hyaline cytoplasm of the megakaryocyte or a larger pseudopod. The granular material in some slender pseudopods may be more or less completely segmented into these rounded masses and such a pseudopod may present the appearance of being composed of a chain of blood platelets united by the continuity of their hyaline ground substance, which in turn, is continuous with the hyaline cytoplasm of the megakaryocyte. The same appearance may be shown by undoubted pseudopods not connected with megakaryocytes and lying in blood channels, and also in the cytoplasm of cells in process of losing their cytoplasm.

The identity in appearance of the small portions of cytoplasm containing the separate groups of granules, and of the small pseudopods, with the various forms of blood platelets in every respect except that they are in continuity with the megakaryocyte, and the occurrence of such separate groups of granules in giant cells in process of losing their cytoplasm, have led me to the conclusion that such small portions of giant cell cytoplasm, and also the small pseudopods, by separation from the cell, become the blood platelets.

Bodies identical in appearance with blood platelets are commonly found near pseudopods, but only rarely are aggregations of free blood platelet-like bodies found associated with a cell in active pseudopod formation. The infrequency of the occurrence of such aggregations of platelets is easily explained by the consideration that by reason of the projection of the pseudopods into blood channels, and because of the close relations of the giant cells to the blood stream in the marrow, the platelets are usually swept into the circulation as soon as separated from the megakaryocytes or their pseudopods. That the megakaryocytes and their fragments have ready access to the blood stream is shown by the occurrence of detached pseudopods in the blood channels and by the well-known fact, first clearly pointed out by L. Aschoff, of

the lodgement of them and of their more or less naked nuclei in the blood vessels of the lung.

The appearances observed are summarized and interpreted as follows:

All of the blood platelets are detached portions or fragments of the cytoplasm of the megakaryocytes, which are in such relation to the blood channels in the marrow that detached portions of their cytoplasm are quickly carried by the blood current into the circulation. The breaking up of the cytoplasm into the platelets occurs only in cells which have reached a certain stage of growth and development, and is probably rapidly completed when once begun. It takes place in various ways but usually by the pinching off of small rounded projections or pseudopods from the cell body or from larger pseudopods, or by the segmentation of slender pseudopods, or by the pinching off of longer or shorter pseudopods which may or may not undergo segmentation later. All or most of the cytoplasm of the giant cell is given off to the blood stream and the nucleus degenerates. The more or less naked nucleus is often carried by the blood stream to the lungs where it lodges in the capillaries. Before the separation of a platelet takes place the red to purple staining granules in that portion of the cytoplasm which is to form the platelet are separated from the rest by a zone of hyaline cytoplasm and arranged in a more or less sharply outlined, rounded or oval mass. The line of cleavage is through this zone of hyaline cytoplasm and this sharply outlined mass of granules becomes the central granular mass of the blood platelet which has been regarded by some observers as a nucleus.

These observations and conclusions concerning the origin and nature of the blood platelets have been confirmed by C. H. Bunting for the rabbit. On the other hand, H. Schridde, who also devised a method of staining the granules in the cytoplasm of the megakaryocytes, could not confirm them for the blood platelets of man, because the blood platelets in his preparations did not show the characteristically staining granules. This must be due to a defect in his staining method, for my method clearly

brings out the same appearances in the megakaryocytes and blood platelets of man as in those of animals.

This conception of the histogenesis of the blood platelets derives additional support from the following considerations:

1. The observation by me, with the aid of Deetjen's method, of protoplasmic movements of identical character both in the hyaline marginal zone of the megakaryocytes and in the hyaline marginal zone of the blood platelets. These movements have been described by H. Deetjen and others for the blood platelets. I have seen the hyaline marginal zone of the megakaryocytes and of the blood platelets constantly changing its outline, sending out and withdrawing short processes of various shapes. This so-called amœboid movement of the blood platelets is not surprising, because it is known that detached fragments of living protoplasm may exhibit movement.

In this connection I may state that I have seen a few megakaryocytes change their form very markedly, sending out and withdrawing pseudopods, such as are seen in the sections. This seems to show that the presence of pseudopods and protoplasmic prolongations of megakaryocytes in blood vessels, as I have seen in the sections, is not a passive act, due to local conditions of pressure in the tissue, but is a manifestation of vital activity. Amœboid activity on the part of the megakaryocytes was suspected by J. Arnold, and has been affirmed by M. Askanazy.

2. A comparison of the numbers of blood platelets per cubic millimeter of blood in certain diseases, as estimated by various observers, with the histological findings in the bone marrow in the same diseases, suggests a relationship between the blood platelets and the megakaryocytes. Thus in pernicious anemia and lymphatic leukemia the blood has been found to contain abnormally few platelets, while the marrow in typical cases of these diseases as far as can be inferred from the reports in medical literature and from my own observations, undergoes profound changes in the character of its cellular constituents with marked diminution in the number of the megakaryocytes. On the other hand, in post-hemorrhagic and secondary anemia the blood platelets are increased in number and there is also increase in the

amount of red marrow with consequent increase in the total number of megakaryocytes in the body. In so-called myelogenous leukemia the blood platelets are also increased in number, and in the cellular accumulations of this disease megakaryocytes do not seem to be an uncommon finding, although but little attention has been paid to them by pathologists. In view of the enormous increase of the marrow cells in this disease it must be obvious that the presence among them of a relatively small proportion of megakaryocytes means a great absolute increase in the number of such cells in the body.

Furthermore, C. H. Bunting has recently shown experimentally on the rabbit that synchronous with or preceding the appearance of an increased number of platelets in the blood stream the megakaryocytes are increased in number.

3. It would seem that the blood platelets do not appear in the embryo before the appearance of the forerunners of the megakaryocytes. Thus in an embryo guinea pig of about 4.5 mm. in length, I have found, free in the blood vessels, cells of about the size of the nucleated red blood corpuscles which have the characteristic staining of the megakaryocyte and differ from it only in being much smaller in size. A study of other embryos shows all grades of transition between this circulatory cell and the typical megakaryocyte. These small megakaryocytes may be seen in the blood vessels in the sections breaking up into typical blood platelets just as do the fully developed cells. (Fig. 17.) On the other hand in a smaller embryo guinea pig I have not found either the small megakaryocytes or blood platelets in the blood.

It is of interest to note in this connection that some at least of these forerunners of the megakaryocytes seem to be formed by a transformation of endothelial cells of blood vessels, because I have seen one of them apparently forming a part of the endothelium of a blood vessel in the yolk sac of a guinea pig embryo. This cell is pictured in Fig. 16.

4. According to my own and others' observations, bodies that are undoubtedly and obviously blood platelets are found only in the blood of mammals, and mammals are the only creatures that have megakaryocytes in the blood-forming organs. I have found

undoubted, characteristically staining blood platelets in the blood of all of a considerable variety of mammals including the elephant, kangaroo, opossum and camel, and I have found megakaryocytes in the blood-forming organs of all mammals including the opossum, which I have examined under satisfactory conditions. The so-called spindle cells or thrombocytes of birds, amphibia, reptiles and fishes have been held by some writers to be the morphological equivalents of blood platelets, but my studies of the blood and blood-forming organs of these vertebrates have not led me to accept this view. I would offer the hypothesis that these peculiar corpuscles are rather the homologues of the megakaryocytes than of the detached fragments of their cytoplasm or the blood platelets, and that these two kinds of cell have been differentiated from one and the same type of cell which circulated in the blood of extinct vertebrates.

This hypothesis is based upon the following considerations:

FIRST. As I have already pointed out, the forerunners of the megakaryocytes are at first circulatory cells in the blood of the embryo guinea pig. This fact points to the megakaryocyte as representing a circulatory cell in the ancestry of the mammals.

SECOND. The spindle cells or thrombocytes of certain amphibian blood have a cytoplasm which stains in the same way as does that of the megakaryocyte; namely, showing a granular red to violet staining endoplasm and a hyaline blue staining ectoplasm, (see Figs. 18 to 21). Furthermore these cells in *Batrachoseps attenuatus* regularly lose their cytoplasm by a pinching off process and the portions thus detached appear as independent corpuscular elements of the blood with great likeness to blood platelets, for they have the same form and outline as blood platelets and the same red to violet staining central portion with vacuole-like spaces in it and the same hyaline blue marginal portion with the irregular or jagged edge. Figs. 18 to 21 show some of the various forms in which these cells appear and some of the platelet-like detached portions of their cytoplasm as well as some of the phases of the process of detachment. The likeness of these detached portions of the cytoplasm of these cells to blood platelets was first pointed out by G. Eisen.

## THE METHOD

The material should be obtained immediately after death or taken from the living animal.

For fixation methyl alcohol, formaldehyde, or a saturated solution of mercuric chloride in a 0.9 per cent solution of sodium chloride, may be used. Methyl alcohol is not now recommended for fixation. Formaldehyde should not be allowed to act longer than forty-eight hours. The method is not applicable to material fixed in Zenker's fluid.

The tissue is dehydrated by alcohol followed by acetone, cleared in thick oil of cedar followed by xylol, and imbedded in paraffin.

The sections should not be more than  $4\mu$  in thickness.

Crystals of corrosive sublimate in the sections are to be removed by treatment with Gram's solution of iodine and alcohol.

The sections are stained while affixed to the slide by Meyer's glycerine-albumin mixture.

The staining fluid and the mode of its preparation are described below.

The staining, clearing and mounting is carried out as follows:

1. Equal parts of the staining fluid and distilled water are mixed in a small wine glass and *immediately* poured on to the slide. The measuring is conveniently done by means of a small pipette provided with a rubber bulb. At least 2 cc. of the freshly diluted staining fluid are thus spread out over the slide, which should be supported upon some object in such a way as to prevent the fluid from running off. The spreading out of the fluid in a layer is important because it facilitates the evaporation of the alcohol whereby the staining elements slowly precipitate out of solution and, while doing so, stain the tissue elements. This precipitate appears as a yellowish metallic scum which slowly forms on the surface of the mixture. The diluted staining fluid is allowed to act for about ten minutes when the preparation is immediately washed in water. The exact time required for the best results has to be determined for each batch of the staining fluid. The proper staining of the preparation may be judged by examining it by a



yellowish artificial light under a low magnifying power after pouring back the diluted staining fluid into the wine glass. The stain is to be regarded as sufficiently intense and the staining process stopped by washing the preparation in water when the cytoplasm of the giant cells has acquired a bright red color and the fibrils of the reticulum begin to take on a red color also. If the staining is found not sufficiently intense the diluted staining fluid is poured back on the preparation and allowed to act longer. Over-staining and the formation of a black red granular precipitate on the preparation occur if the diluted staining fluid is allowed to act longer than a certain time.

2. Dehydrate in pure acetone.

On account of the great volatility of acetone some care is necessary to prevent the drying of the preparation, which should be avoided.

3. Clear in pure oil of turpentine.

4. Mount in a thick solution of colophonium in pure oil of turpentine.

Before mounting the preparation the superfluous turpentine should be carefully removed because this reagent rapidly takes up water from the air and thus may cause the clouding of the preparation or the fading of the stain.

The solution of colophonium is made by saturating a quantity of turpentine with powdered colophonium and keeping the filtered solution in the paraffin embedding oven until it has evaporated to the required consistence.

The use of acetone for dehydrating and of oil of turpentine for clearing and mounting is an important feature of the method, for these do not destroy the characteristic staining of the granules in the giant cells and platelets as do other similar reagents that I have tested.

The staining fluid is composed of a mixture of 3 parts of a solution of modified or polychromatized methylene blue and 10 parts of a 0.2 solution of eosin, "w.g." (Gruebler) in pure methyl alcohol. It is permanent if kept in a well-stoppered bottle so that evaporation is prevented.



The solution of methylene blue is prepared as follows: One gram of methylene blue, B. X. (Gruebler) is dissolved as thoroughly as possible in 100 cc. of a 0.5 per cent aqueous solution of sodium bi-carbonate in an Ehrlenmeyer flask. The flask and its contents are then placed in an ordinary steam sterilizer and kept at 100°C. for one hour and a half, counting the time after the steaming has become vigorous. When cool, the mixture is filtered and the filtrate is the modified blue solution. It must be of a well-marked purple color when viewed in a thin layer by the yellow transmitted light of an ordinary incandescent electric bulb. This color appears only after cooling.

It is important that the quantities mentioned should be accurately weighed or measured. An excess of eosin delays the appearance of the scum on the surface of the diluted staining fluid and the time required for staining will be longer than ten minutes. On the other hand, an excess of the modified blue component hastens the appearance of the scum and the staining may in ten minutes cause over staining and the granular precipitate to form on the preparation.

The preparations should be viewed by the light from an incandescent electric bulb which has a yellowish tint. This brings out more strongly the characteristic color of the granules in the megakaryocytes and in the blood platelets.

My thanks are due to Prof. S. H. Gage of Cornell University and to Prof. C. S. Minot, Dr. F. T. Lewis and Dr. J. L. Bremer of the Harvard Medical School for material for study. I am especially indebted to Dr. J. W. Dewis of Boston for a collection of preparations of the blood of various animals, the study of which first awakened my interest in the subject of the histogenesis of the blood platelets and was the starting point of the work upon which this paper is based. To Dr. Oscar Richardson, Assistant Pathologist, I am under many obligations for relieving me of much of the routine work of the Laboratory during the progress of this work.

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## EXPLANATION OF FIGURES

The water color drawings were made by the author with the aid of a camera lucida. They are all drawn at the same magnification, except fig. 6, which is drawn with a 3mm. Zeiss apochromatic oil-immersion objective, while the others are drawn with a 2 mm. objective of the same kind. The eye-piece used in all cases was compensating ocular 6. With the exception of figs. 18 to 21 all the figures were drawn from sections either of the bone marrow or spleen of the cat or kitten.

FIG. 1. Megakaryocyte in position of amœboid activity with its protoplasm projecting far into the lumen of a blood vessel in the form of pseudopods. Blood platelets free and in process of segmentation from the pseudopods. Vacuoles in cytoplasm and in platelets.

FIGS. 2-4. Megakaryocytes which have lost nearly all of their cytoplasm and present more or less degenerate nuclei. Platelets are shown free and in process of budding off from the cytoplasm. Vacuoles in cytoplasm and platelets.

FIG. 5. Megakaryocyte showing excessive development of pseudopods with blood platelets developing from them.

FIG. 6. Megakaryocyte with its cytoplasm prolonged into a blood channel in the form of a very long pseudopod. The continuity of the different portions of

the pseudopod was shown by serial sections. At the right lower corner of the drawing are shown three blood platelets and a lymphocyte. The other small cells in the vessel are leukocytes.

FIG. 7. Megakaryocyte in the act of protruding a pseudopod through the wall of a blood vessel into its lumen. At the extremity of the pseudopod three platelets in process of development. Further down in the blood vessel four free platelets. Vacuoles in cytoplasm and in some platelets.

FIG. 8. Detached pseudopods projecting into a blood vessel and in process of segmentation into platelets. Free platelets also shown. Vacuoles in cytoplasm and in platelets.

FIG. 9. A mass of cytoplasm of a megakaryocyte with platelets budding off from it. Free platelets and different forms of white blood corpuscle also shown.

FIG. 10. Detached pseudopods and blood platelets. One of the pseudopods segmenting into blood platelets.

FIG. 11. Same as fig. 10, except that one of the larger granular red masses is in continuity with a giant cell not obvious in the section.

FIG. 12. Megakaryocyte protruding a pseudopod into a blood vessel through an opening in its wall. At the tip of the pseudopod two or three platelets in process of development and two or three developed platelets. The arrangement of the granules in rows is shown.

FIG. 13. Megakaryocyte showing a platelet in process of pinching off from a pseudopod protruding through the wall of a small blood channel.

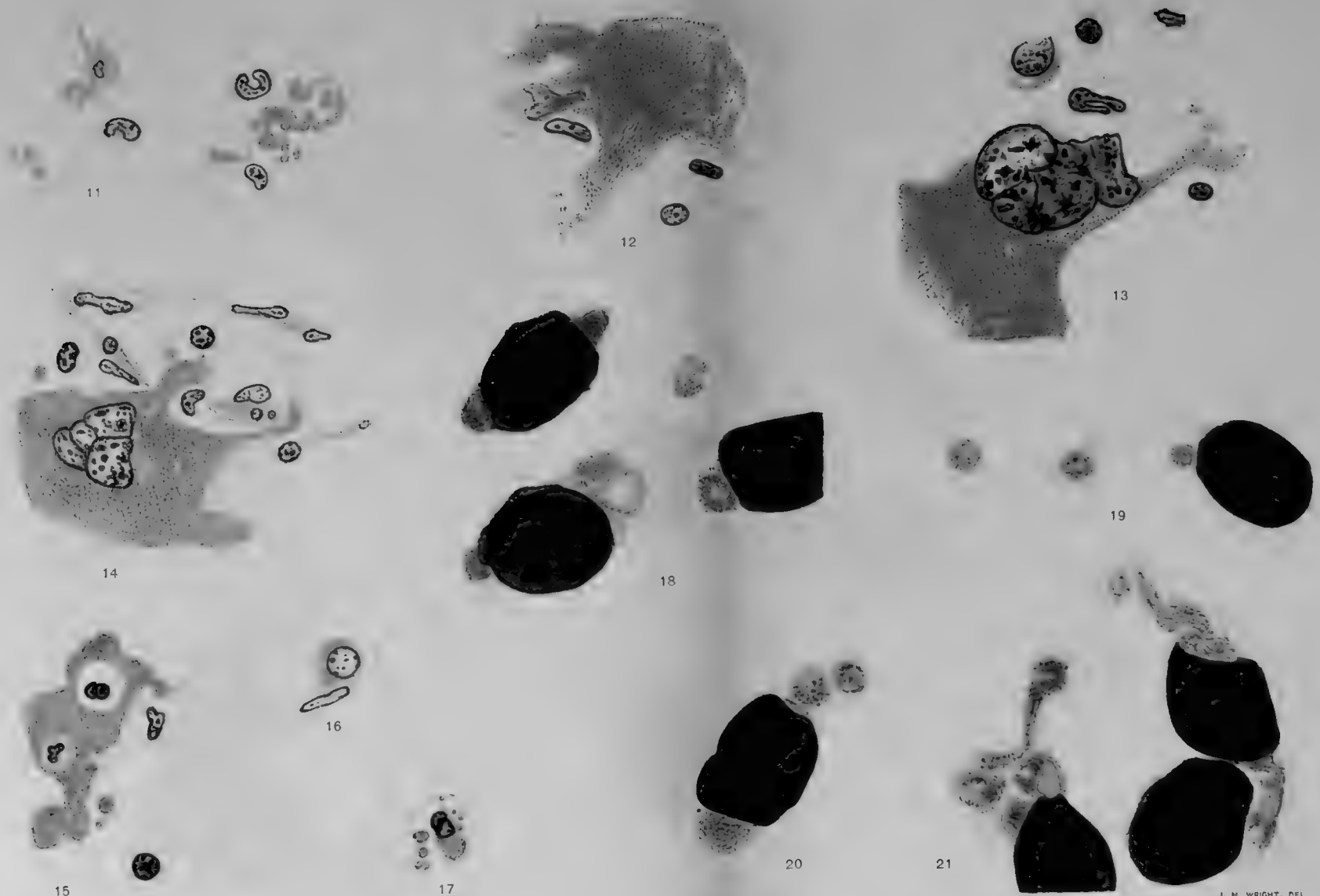
FIG. 14. Megakaryocyte extending two pseudopods into a blood vessel through openings in its wall. A blood platelet pinching off from one of them. Two blood platelets free. Vacuoles in the cytoplasm of the giant cell are shown.

FIG. 15. Megakaryocyte with a blood platelet in process of budding off into a small blood channel. Two other blood platelets in the lower part of the figure.

FIG. 16. One of the forerunners of the megakaryocytes observed in the blood of a young guinea pig embryo. This cell is apparently in process of development out of an endothelial cell of a blood vessel. It still forms part of the endothelium of the wall of the blood vessel and it clearly is a transformed endothelial cell.

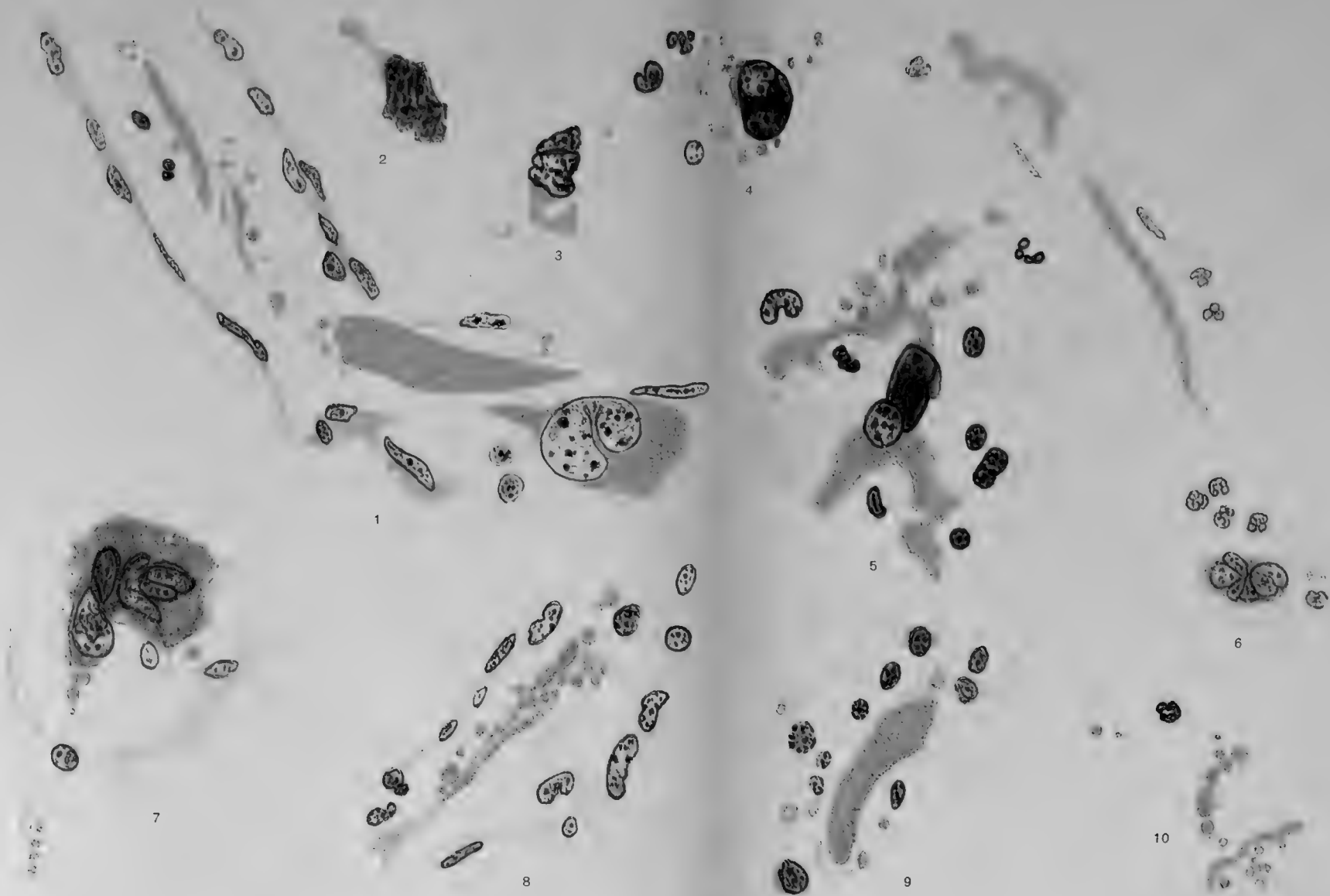
FIG. 17. One of the forerunners of the megakaryocytes in the blood of an early guinea pig embryo in process of breaking up to form blood platelets. Free blood platelets also are shown.

FIGS. 18-21. Spindle cells or thrombocytes of *Batrachoseps attenuatus* and free blood platelet-like corpuscles. Smear preparations stained by Wright's blood stain. Various phases are shown in the process of pinching off portions of the cytoplasm of the thrombocytes to form blood platelet-like corpuscles. The thrombocyte in fig. 18 has lost nearly all its cytoplasm by the process of pinching off of platelet-like fragments. The yellow body in one of the cells in fig. 21 is a fragment of a red blood corpuscle that has been taken into itself by the cell. Note the vacuoles in the platelet-like body in fig. 19 and in the pseudopods in fig. 21.



J. M. WRIGHT, DEL.







# FURTHER STUDIES ON REPRODUCTION IN SAGITTA

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WITH ONE HUNDRED AND TWO FIGURES

## INTRODUCTION

In March, 1905, it was my privilege to avail myself of the opportunity offered by the newly opened winter quarters of the Marine Biological Laboratory at Woods Hole, Mass., to make a further study of the method of egg-laying in *Sagitta elegans*. A recent paper ('05) had described, as fully as was possible from fixed material alone, the later stages in the ripening of the ovum and its entrance into the oviduct which is temporarily opened up between the sperm-duct and the median oviduct wall. Questions arose as to the relation of the two ducts at the point of opening to the exterior, and also as to the activity or passivity of the ovum in its passage from the ovary to the reproductive pore.

Hertwig ('80) in "Die Chætognathen," (pp. 52-54 and Pl. 4, Fig. 13), describes and figures the sperm-duct as the oviduct, and the oviduct wall as the 'Keimlager.' He observed living spermatozoa in the 'oviduct' but never in the ovary. Finding no opening from the ovary to the duct, he concluded that the eggs when ripe must enter the duct at its posterior end, and that the anterior portion of the observed duct must serve merely as a 'Samentasche.' This duct had been previously described by Krohn ('53) and by Leuckhart and Pagenstecher ('58) as a 'Samentasche' Kerferstein ('62) also saw spermatozoa in this canal, but nevertheless regarded the whole of it as an oviduct, and was of the opinion that there must be an anterior opening from the ovary into the oviduct. Grassi ('83) called the duct a sperm-



oviduct, but did not discover by what method the eggs escaped from the ovary.

In two brief papers ('03, '05) I have shown that the duct described by previous investigators of *Sagitta*, is a sperm-duct (Samentasche), or sperm-receptacle, lying within the oviduct which is closed except when ripe eggs are being discharged. ('03, Figs. 1-3; '05, Figs. 1, 11, 12). The 'Keimlager' of Hertwig, which forms the wall of the oviduct, I had suspected was not germinal epithelium at all and therefore not a part of the ovary proper, or only an accessory structure to be classed with the sperm-duct and the endothelial membrane covering the ovary, and not with the germ cells. This was one of the points which led to further investigation of the reproductive system of *Sagitta*.

#### OBSERVATIONS ON LIVING MATERIAL

Through the kindness of Mr. George M. Gray, curator, I was able to sample the *Sagitta* material at intervals during March, 1905, and thus to time my arrival at Woods Hole when the laying season was just beginning.

The time of day when *Sagitta elegans* discharges its eggs is not as definite as in the case of *Sagitta bipunctata*. The latter lays about sundown, while the former has been observed to discharge its eggs at various times between 11:00 a. m. and 6:00 p. m., and there is no reason for thinking that the eggs may not be laid at any other time in the twenty-four hours.

In *Sagitta* collections brought in at about 9:00 a. m. all stages of egg-ripening were found in different individuals. The nuclear membrane disappears from 15 to 30 minutes before the egg begins to push its way into the oviduct. That the egg does actively push its way between the oviduct wall and the sperm-duct, and by its own contractions or by shifting of material within the egg-membrane, make its way down the oviduct to the reproductive opening, I have no doubt, after observing the process in many individuals.

Fig. A is a freehand sketch from a living *Sagitta* under a low power of the compound microscope, showing five eggs entering

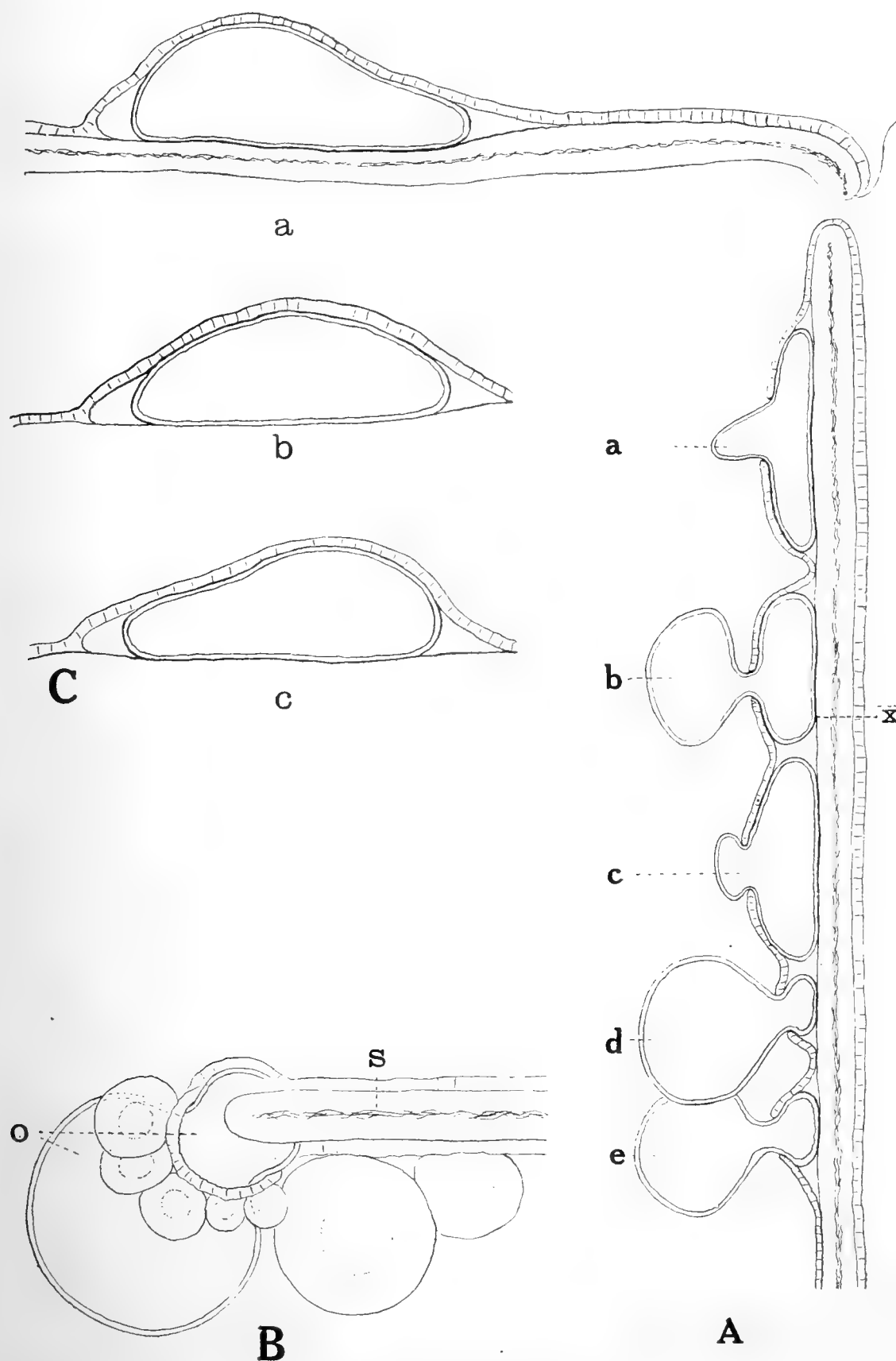


Fig. A. Freehand sketch of eggs entering the oviduct.

" B. Egg entering anterior end of a closed oviduct.

" C. a, b, c. Successive form changes of an egg moving down the oviduct.

one oviduct, as observed at 10:00 a. m. At 10:10 the egg *a* was wholly within the oviduct, at 10:15 *c* was in, and 11:00 all five were in, and *a* had moved down to *x*. Two eggs in the other ovary were still partly outside of the oviduct. In every case observed each egg made its way through its own opening into the oviduct, forcing apart the sperm-duct and the anterior wall of the oviduct. Fig. B is a sketch showing a ripe egg at the anterior end of an ovary, observed at 11:30 a. m., forcing an entrance into an otherwise wholly closed oviduct. The sperm-duct (*s*), containing live spermatozoa lies above the ripe ovum (*o*) and the younger oöcytes.

In some cases a number of eggs—as many as nine—were crowded into one oviduct, occupying its whole length; in others a single egg was seen to pass down the whole length of the oviduct alone. In the latter case the egg was often drawn out in sausage shape so that it extended half the length of the ovary or more. One especially favorable egg was watched from 4:00 p. m. until it was extruded at 5:25 p. m. When first observed, it appeared as in Fig. Ca. Sketches were made at intervals of about 45 seconds, and though there was constant change in the form of the egg, the changes showed a regular rhythm, running from *a* through *b* and various intermediate forms to *c*, in from  $2\frac{1}{4}$  to 3 minutes, and then repeating the series of form changes. With each *a*-phase the egg made a slight advance down the oviduct. There was no visible muscular movement of the body corresponding to the various form changes observed in the egg. In Fig. Ca an air (?) space is shown in the oviduct at each end of the egg. Such a space is always observable in the case of a single egg in the oviduct, in both living specimens and in sections. The first muscular movement observed occurred when the advance air-bubble reached the reproductive pore. The pore suddenly opened wide, the air-bubble, covered by a thin film, passed out and broke, and the egg followed, the opening closing behind it (Fig. Da and b). Both opening and closing had the appearance of a reflex. When several eggs were passing, crowded together, down the oviduct, the pore was only partly closed and then opened wide for another egg. Several times the sphincter closed too soon and cut an egg in two.

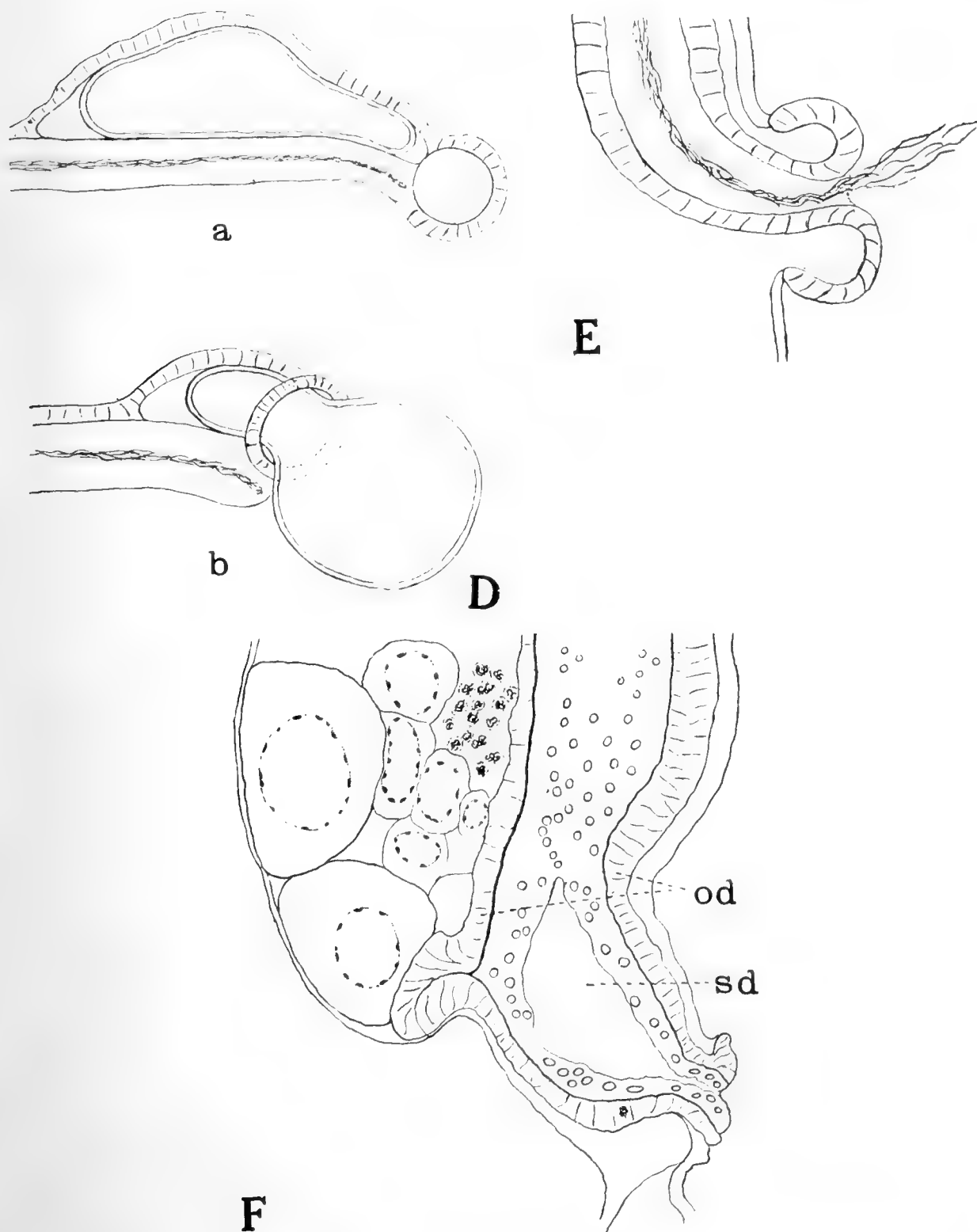


Fig. D. *a, b.* Opening of the exterior sphincter of the oviduct, and escape of the egg.

Fig. E. Spermatozoa escaping from the sperm-duct under pressure on the cover-glass.

Fig. F. Longitudinal section of ovary, oviduct, and sperm-duct.

This was very likely due to the abnormally slow passage of the egg under laboratory conditions.

Both living material and sections show that the sperm-duct and oviduct are entirely separate for their whole length, each having its own opening to the exterior. By exerting pressure on the cover-glass, a stream of sperm was forced out of the mouth of the sperm-duct when the oviduct, including its sphincter-like pore, was entirely closed. This is shown in Fig. E, an optical

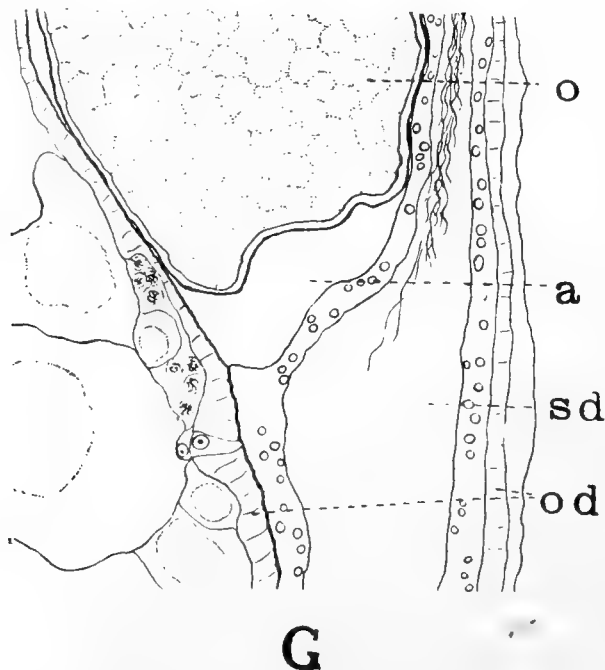


Fig. G. Longitudinal section of an egg in the oviduct.

section of the sperm-duct with its external opening, and the walls of the oviduct adhering to the sperm-duct as they normally appear except when eggs are being discharged. In Fig. F, a camera drawing from a longitudinal section of *Sagitta elegans*, the same conditions are shown. The sperm-duct (*sd*) is here much enlarged but empty, and the oviduct (*od*) wall shows a characteristic fold which opens, in part at least, when an egg passes. In Fig. G, the sperm-duct contains spermatozoa and the oviduct is being opened up by an advancing egg (*o*), which pushes apart the sperm-duct and the anterior wall of the oviduct. An air-space (*a*) appears here as in the living material.

These observations make plain the relation of the two ducts to one another and the activity of the egg in reaching the exterior when ripe. Study of a very complete series of sections of *Sagitta* from the beginning of post-larval life to maturity seemed to be necessary to settle satisfactorily the origin and development of these ducts. I had no opportunity to secure such material until the summer of 1909, when, through the courtesy of the directors of the marine laboratories at Pt. Erin, Isle of Man, and at Helgoland, I obtained an abundance of *Sagitta bipunctata* in various stages. I am especially indebted to Mr. Chadwick, curator at Pt. Erin station, for assistance in collecting and fixing material, as well as for many other courtesies. This material was studied while I was enjoying the privilege of working in the Zoölogisches Institut at Würzburg, Germany. Later, eggs, embryos, and young *Sagittas*,  $7\frac{1}{2}$  days old, were studied at Naples.

#### ORIGIN AND DEVELOPMENT OF THE DUCTS

Hertwig's well-known figures show the four primary germ cells free in the coelomic cavity of the gastrula, later embryo, and recently hatched young *Sagitta*. In the 10-day old *Sagitta* he figures the two pairs of primary germ cells attached to the body-wall, and each cell covered by a layer of endothelium. In my  $7\frac{1}{2}$ -day larvæ of *Sagitta inflata*, I find the germ cells not yet covered by endothelium, although the endothelial cells in some cases appear to be creeping up over them. Figs. 1 and 2, are longitudinal optical sections through the germ cells of whole mounts stained with borax carmine, and Fig. 3 is a cross section through one of the two primary oögonia ( $7\frac{1}{2}$  days). My later stages are all *Sagitta bipunctata*. The youngest, obtained from plankton at Naples, have from 4 to 7 cells in each ovary, and show in a single section (Fig. 4) two or three oögonia lying against the body wall and covered by a layer of endothelium. In ovaries containing from 16 to 20 oögonia the antrum has begun to form below the germ cells. Figs. 5 and 6 are sections of the two ovaries of the same individual, showing the same conditions as in Fig. 4,—a group of oögonia covered by a layer of endothelium, which is continuous with the

lining of the body wall. Just below the oögonia, a group of cells (Fig. 7) with a small lumen was found; this is evidently the beginning of the antrum, of which the oviduct is a cephalad continuation. The section below shows a strand of cells running from the body wall to the transverse membrane which separates the two body cavities. Figs. 8 to 11 were taken from a somewhat older ovary containing 35 to 40 oögonia. Fig. 8 is the most posterior section of the ovary proper, showing a single oögonium surrounded by cells which will later give rise to the antrum and ducts. The section below (Fig. 9) shows more of the antrum cells in the form of a fold or outgrowth of the inner or mesodermal layer of the body wall. (Cell outlines were not distinct.) The next section below contained the remainder of these cells, a thin strand extending to the transverse membrane. Fig. 10, the third section above Fig. 8 and Fig. 11, the tenth above Fig. 10, show nothing more than Figs. 5 and 6; i.e., no ducts yet formed. If, however, we take a considerably later stage where the growing oöcytes can be distinguished from the oögonia (Fig. 12), we find in addition to the endothelial membrane, two other layers of cells between the body wall and the germ cells. These layers extend around the sides of the ovary forming a 2-layered crescent (*od*). There is as yet no lumen and no sperm-duct.

Fig. 13 shows the next stage, in which a different kind of tissue (*sd*) has come in between the two layers of the lateral wings of the future oviduct. This tissue evidently must either be proliferated from the layers which surround it or be formed by migration from those layers, as there is no other possible source. No cell outlines can be distinguished, the tissue appearing to be a syncytium of a fibrous nature with scattered nuclei. In this stage mitosis is frequent in the oviduct walls, but rare in the new tissue between them. Fig. 14 is a somewhat older ovary where the oviduct walls are completely separated in the middle by the meeting of the new tissue, which first appeared in the wings. In Fig. 15 we have a section of the lower or caudad end of the ovary, showing the oviduct walls continuous with the as yet closed antrum. The reproductive pore is formed, but the cells have not separated to form the antrum chamber and external opening.

The sperm-duct tissue has not appeared in the antrum region. By comparing this figure with Fig. F, one can easily see the relation of the embryonic layers to the adult oviduct and sperm-duct. In one case only, shown in Fig. 16, have I ever seen at this stage the sperm-duct tissue separated from the median oviduct wall, leaving a lumen corresponding to the space through which the ripe eggs pass to the exterior.

Somewhat later the whole oviduct with the contained sperm-duct tissue contracts laterally, as shown in Fig. 17, changing the flat layer of sperm-duct tissue into an approximately cylindrical rod of syncytial tissue with nuclei rather irregularly scattered and vacuoles here and there (*v*). Figs. 18 and 19 show the appearance of an irregular lumen, and Fig. 20 the presence of spermatozoa in the more regular lumen of an older duct. In Fig. 21 we have a longitudinal, but slightly oblique, section through the reproductive opening and antrum of an adult *Sagitta*, showing the relation of the two ducts and their openings when the sperm-duct is slightly open and the oviduct closed. In Fig. 22 is shown a transverse section of an ovary of *Sagitta bipunctata* with a ripe egg in the oviduct, to the outer wall of which the sperm-duct (*sd*) remains attached. The walls of the oviduct are much stretched, but the lateral wings are not opened as one might expect them to be.

Thus we have traced the development of the oviduct from its origin in a fold of mesoderm at the base of the ovary, and the formation within it of a sperm-duct or 'Samentasche' with a separate opening from that of the oviduct. These ducts are without doubt purely accessory mesodermal structures, and have no direct connection with the germ track of *Sagitta*. In adult ovaries the young oögonia and youngest oöcytes are sometimes crowded against the median wall of the oviduct, and are even pressed in between the cells in such a manner that they appear to be a part of it, but I am convinced that this is only a secondary appearance and that there is always a portion of the oviduct wall between the germ cells and the sperm-duct.

The oviduct wall furnishes two accessory fertilization cells to each ovum, and the lateral wings may be largely reserve cells to make good the loss from this source. The central ends of the cells of



the oviduct wall stain as though they contained supporting fibers or possibly muscle fibers, though in watching the passage of eggs down the oviduct I have been unable to detect any muscular activity in the oviduct walls. I have thought that these fibers might be of an elastic nature, but have not tested their staining reaction with this point in view. They stain deeply with iron-haematoxylin, but so does any particularly dense portion of a cell. The stretching to which the walls of the oviduct are subjected when eggs are passing through it naturally suggests that the cells may have developed material of an elastic or supporting nature to meet this strain.

#### MATURATION AND SEGMENTATION OF EGGS IN THE OVARY

While making the above observations on egg-laying in *Sagitta elegans*, it was noticed that in several individuals asters were present in eggs which were either free in the ovary or were in the oviduct. The first cases noted were in material which had been kept in the laboratory over night, but later the same phenomenon was observed in individuals fresh from the sea. Several such specimens were fixed and sectioned. Eggs were found free in the ovary in all stages of maturation and segmentation up to an 8-16-cell stage. Fig. 23 shows the first polar body and the second polar spindle of such an egg, and Fig. 24 the uniting male and female pronuclei. Figs. 25 and 26 are the first and second segmentation spindles in metakinesis and anaphase respectively. These figures make it plain that development here is that of fertilized eggs and not a case of parthenogenesis. Fig. 27 is a section of an 8-cell stage from an ovary in which several segmenting eggs were found. At present I have no satisfactory explanation to offer for this premature development of *Sagitta* eggs. Nothing of the kind was seen, either in material from Naples or in that from Woods Hole in 1904, the only approach to it being the cases of two polar spindles in eggs of one individual described in "Further Studies on the Ovogenesis of *Sagitta*" ('05, p. 246, Figs. 11, 15, 16, and 17). Whether these segmenting eggs could ever be laid seems doubtful, for the moment of breaking away from the oviduct

wall seems to be the opportunity to open a way to the exterior. Failure to make their way into the oviduct may be the whole explanation of the conditions observed. The eggs are already fertilized and therefore ready to go on developing unless some added stimulus from the sea-water were necessary to cause them to complete their maturation and begin to segment. That this is not the case is evident; after the egg is fertilized and has broken away, maturation and the early segmentation stages go on whether the egg is laid at the normal time or is retained in the ovary. Most of these eggs had decidedly irregular outlines suggesting amœboid movements and supporting my conclusion that the eggs make their way into and down the oviduct by their own activity.

#### ELPATIEWSKY'S 'BESONDERE KÖRPER'

The appearance of Elpatiewsky's ('09) paper on "Die Urgeschlechtszellenbildung bei Sagitta" invests these segmenting eggs with a new value. Elpatiewsky finds in the development of normally laid eggs a stainable body which, from the first segmentation on, marks the germ track; and, possibly, is in the sixth division the determining factor in the separation of the germ plasm into primary oögonia and primary spermatocytes. This 'besondere Körper,' as Elpatiewsky designates it, is first seen near the vegetal pole of the egg when the two pronuclei are in the center of the egg, and the first cleavage plane passes a little to one side of it. Although Elpatiewsky states that he first finds this homogeneous stainable body in the stage where the two pronuclei are uniting, my first thought on reading the paper was that this body must have some relation to the accessory fertilization cell which I had described ('05) as degenerating after the egg breaks away from the oviduct wall. This description was based on the degenerate appearance of the cell in eggs nearly ready to break away, and on the fact that I had been able to find no trace of it in the eggs after they entered the oviduct. Indeed, I suspected that in some cases the accessory cell might be actually pulled out of the egg.

I had no difficulty in finding Elpatiewsky's 'besondere Körper' in the free eggs of *Sagitta elegans*, provided that they had reached the stage when the two pronuclei were in the center of the egg, as in Fig. 24. Fig. 28 shows this body from the same egg as Fig. 24, and Fig. 29 *a* and *b* is another similar stage, while Figs. 30 and 31 are sections from 2-cell and 16-cell stages. It was also found in all stages between those of Figs. 29 and 31, but when I came to look for it in earlier stages, of which I had an abundance, both of *Sagitta elegans* and of *Sagitta bipunctata*, no connection between the 'besondere Körper' and the accessory fertilization cell could be traced. Figs. 32 *a* and *b* show an egg in which the accessory cell (*a*) is evidently degenerating, and the nucleus of the egg is breaking down preparatory to the first maturation mitosis; Figs. 33 *a* and *b* another egg, still attached, with the first maturation spindle in prophase. In the latter case the nucleus of the accessory cell looked quite normal, while in the former the whole cell stained dark and a somewhat lighter portion in the center of the cell was the only indication of the disappearing nucleus. In general it may be said that at this stage the accessory cell looks shrunk and dark, and the nucleus has either degenerated or is on the point of doing so. Fig. 34 shows an egg in which the two accessory cells were both much shrunk when the first maturation spindle was in metaphase. In such a stage as Fig. 32 *a* the accessory cell is about the same size as the 'besondere Körper' (Fig. 29) and both stain deeply. The accessory cell, however, has a distinct cell boundary and the 'besondere Körper' quite a different structure, being composed of a non-staining homogeneous matrix filled with deeply staining granules. Fig. 35 shows one case where this body was not spherical but irregular in outline, consisting of somewhat scattered masses, at a stage when the first segmentation spindle was in metaphase.

In maturation stages between Figs. 32, 33, 34, and Fig. 29, I find absolutely no trace of either accessory fertilization cell or 'besondere Körper.' This is in entire agreement with the conclusion of Elpatiewsky, and is based on examination of many preparations of *Sagitta elegans* (1904 material) in which eggs containing the first maturation spindle in metaphase were passing down the ovi-

duct, and also on many preparations of the same species containing eggs developing free in the ovary (1905 material). Several preparations of *Sagitta bipunctata* with eggs in the oviduct were examined with the same result.

In one egg of *Sagitta elegans*, containing the second maturation spindle, I did find, near the vegetal pole, a spot of denser cytoplasm (Fig. 36 *a* and *b*) which, however, was not at all stained with iron-hæmatoxylin. This may have been an early stage of the 'besondere Körper,' but could, I think, hardly be a transition stage between the degenerating accessory cell and the 'besondere Körper,' since degenerating nuclei nearly always stain dark.

It also seemed possible that the granules of chromatin-like material extruded from the nucleus in preparation for maturation of the egg ('03, Pl. 1, Fig. 2 *b*), a phenomenon which is even more marked in *Sagitta elegans* (Fig. 37), might have some part in the formation of the 'besondere Körper,' but apparently these granules all disappear completely before this peculiar body becomes visible. At the stage shown in the two figures referred to, small black granules are scattered all through the nucleoplasm and a quantity of larger granules are found outside the membrane between the nucleus and the point of attachment of the egg. If either these granules or the accessory fertilization cell take part in the formation of the 'besondere Körper,' it must be after an intermediate stage in which the material does not stain.

In a recent paper, Buchner ('10) traces Elpatiewsky's 'besondere Körper' to the degenerate nucleus of my 'accessory' cell, but curiously enough he describes the recently laid eggs as containing "1) das Degenerat der Strangzellen, 2) das Spermium, zwar schon mit einem Strahlenhof umgeben, aber noch nicht zum männlichen Vorkern aufgequollen, 3) die Telophase der ersten Reifenteilung des Eikerns," and then he refers to his Fig. 5, which is not the stage which he describes but the one where both Elpatiewsky and I first find the 'besondere Körper.' (Compare Buchner Fig. 5, Elpatiewsky Fig. 3, Stevens Figs. 24, 28, 29).

The "mit Eisenhæmatoxylin sich tief schwärzendes Netz" to which Buchner refers and upon the importance of which he lays considerable stress in connection with his hypothesis of the chromi-

dial nature of the 'besondere Körper' and of the nucleolus-like bodies of the oögonia and oöcytes, I have spoken of in describing the development of the oviduct. I find no such connection with the 'accessory' cell as he figures on page 435, and a careful review of all my preparations of material from four species has only served to confirm my previous account of the appearance and function of the two epithelial cells which connect each oöcyte with the sperm-duct.

#### THE ACCESSORY FERTILIZATION CELLS

Fig. 38 shows an exceptionally good section of the accessory or connecting epithelial cells in *Sagitta bipunctata*. The fertilization canal, which Buchner says he has not been able to find, is shown, and the spermatozoön (s) is partly in the second cell. It is only occasionally that one finds the spermatozoön in the cells, but when found, it is not to be mistaken for any such connecting fiber as Buchner figures. In the broad portion of the canal there is a large, granular non-staining spindle-shaped body (b) which may, I think, be some substance attractive to the spermatozoa. This I first saw in living eggs while attempting to observe the entrance of a spermatozoön into the fertilization canal. In the living eggs both connecting cells can be distinguished, though their nuclei are invisible and the canal extending through both cells is plainly seen, as is also the spindle-shaped body in the widest part of the canal. Fig. 39 is a camera sketch from such a living egg of *Sagitta inflata*, with the nucleus visible but near the periphery of the egg, indicating the approach of maturation. Many spermatozoa were around the mouth of the canal, but I have never been able to detect one in it, possibly because I have used too late a stage for observation.

Fig. 40 shows another case where a part of a spermatozoön is seen in a section of the canal in the inner accessory cell. Figs. 41a and b show the same cells from an egg of *Sagitta minima*. Here the fertilization tube (c) is coiled in the outer cell. This I also observed in the living egg. Fig. 42 is a section through these cells in *Sagitta elegans* showing several portions of the more or less

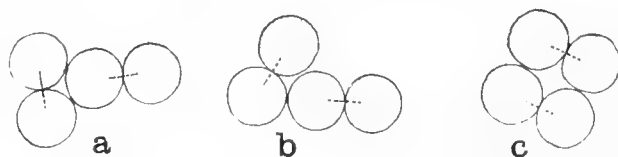
winding fertilization canal (*c*). The eggs of *Sagitta elegans* have a much thinner membrane than those of *Sagitta bipunctata*. In this figure one sees the usual conditions in the region of attachment of an egg which is nearly ripe,—the ends of the surrounding cells of the oviduct wall stain more deeply, and one also finds the stain showing between cells, simulating fibers. The nuclei of two cells adjoining the outer connecting cell have perfectly black nuclei, probably indicating degeneration. In Fig. 43 is shown the place where an egg has broken away without making its way into the oviduct. The cells surrounding the torn connecting cell (*a*) stain almost perfectly black on a well differentiated slide. It is also true that when an egg is passing from the ovary into the oviduct the cells on the border of the opening through which the egg is passing stain deeply, indicating some change connected with the ripening of the egg. In *Sagitta inflata* the nuclei of the connecting cells stain darker, and it is more difficult to say how early degeneration begins. Fig. 44 shows the two connecting or accessory cells of a comparatively young egg and Fig. 45 of an older egg. In both figures sections of the fertilization tube (*c*) are seen.

#### THE 'BESONDERE KÖRPER' IN THE PRIMARY GERM CELLS

My material does not include the stage in which the 'besondere Körper' divides in the sixth segmentation mitosis, which gives rise to the two primary germ cells, but I find the remains of this body in many sections of young gastrulæ. In *Sagitta bipunctata* they are very often conspicuous in a stage where the germ cells are still buried in the gastrula wall (Fig. 46 *k*). In this particular case these bodies were as deeply stained as in the earlier stages. When the two germ cells are in mitosis, I find only less deeply staining fragments (Fig. 47 *a* and *b*, *k*). Likewise in the 4-germ cell stage I find some fragments (Fig. 48). Corresponding bodies are found in *Sagitta inflata* and *Sagitta elegans*, but in both they are less conspicuous. Fig. 49 is an early 2-germ cell stage of *Sagitta elegans*, showing one gray mass in cell *a*, none in *b*; in the next section there were two more such masses in *a*, but none in *b*, indi-

cating that the fragments disappear somewhat earlier than in *Sagitta bipunctata*. Figs. 50 and 51 show the remains (*k*) of the 'besondere Körper' in *Sagitta inflata*; here it is granular and yellowish, not taking the hæmatoxylin. My sections all indicate, as Elpatiewsky says, that division of the 'besondere Körper' is unequal, a larger portion going to one of the first two germ cells than to the other.

As to whether this body is a special device for determining sex in a hermaphrodite organism, I do not think we can safely express an opinion without more evidence drawn from the embryology of other hermaphrodite forms. The special object of my renewed



## H

Fig. H. Freehand sketches showing relative positions of the two pairs of primary germ cells.

study of the germ cells of *Sagitta* at Naples last year was to see whether the division of the first two primary germ cells to form two primary oögonia and two primary spermatogonia is in any way a visibly differential mitosis. I have not been able to detect anything of that nature. Before Elpatiewsky's paper appeared, I had noted the fact that the two primary germ cells never divided synchronously. One finds one cell in prophase, the other in metaphase; one in metaphase, the other in anaphase; one in anaphase, the other in telophase, etc. Having also noticed that the arrangement of the four germ cells in a row is a secondary matter, I had suspected that the differential division might be the earlier one. When the two cells are in mitosis, the two spindles in metaphase often stand nearly at right angles, and one finds the four cells soon after this mitosis in various positions (Fig. H *a*, *b*, and *c*). In the older gastrulæ, after the entoblast folds are formed, the four cells usually form a nearly straight row, as in Fig. 51. Fig. 52 shows the four germ nuclei in an egg of *Sagitta* in-



flata, as one looks down into the gastrula cavity. Fragments of the 'besondere Körper' are found only in the two cells with the smaller nuclei which are the products of the later mitosis. In the stage shown in Fig. 51, I am unable to detect any constant difference in either cytoplasm or nuclei of the cells, and even in young ovaries and testes consisting of a single cell (Figs. 1 to 3) or containing a number of oögonia or spermatogonia, the cells are so much alike that one must depend upon other anatomical features to determine whether one is looking at a section of a young ovary or a young testis.

#### INTRANUCLEAR GRANULES AND NETWORK

In my 1903 paper I referred briefly to certain large black granules found on the inside of the nuclear membrane of young oöcytes, and also to the "reticular network" conspicuous in the nuclear membrane of all older oöcytes up to the time when the egg membrane forms. While I was working on the development of the sperm-duct and oviduct in Professor's Boveri's laboratory, he suggested that it might be interesting to investigate the origin of this network in view of its possible relation to the mitochondria recently described in the cytoplasm of many kinds of cells. At the time I could not trace it back farther than the young oöcytes, perhaps a little younger than those shown in Figs. 4 and 6, Pl. 1, '03, and I thought it desirable to examine more carefully than I had previously done the embryonic and larval stages of the germ cells, with this question in mind. Sections of those, however (Figs. 1 to 3, 46 to 52), throw very little light on the point. The embryonic germ cells show some granules, but nothing really comparable to those of the young oöcytes. The oögonia of young ovaries show several rather large granules, or chromatin-nucleoli, against the nuclear membrane (Figs. 8 and 10). Dividing oögonia also show these nucleoli outside of the spindle in both metaphase (Fig. 53) and anaphase (Figs. 54 and 55). In the daughter nuclei no such nucleoli are found, and it therefore seems probable that these chromatin-nucleoli consist of material thrown out by the chromosomes in the interval between two mitoses, and dissolved



in the cytoplasm during or after each mitosis. In Fig. 56, *b* is a cell in prophase and *a* two recent products of mitosis. The resting nuclei of oögonia often show as many granules in tangential section as in Fig. 57 or 58, but it is in the young oöcytes at the very beginning of the growth stage that one finds relatively large masses of this chromatin-like material inside the nuclear membrane. It most often lies at the two ends of an elongated nucleus, and several of the chromosomes are closely associated with it (Fig. 59). In Fig. 60 one of these masses has begun to divide.

Figs. 61 and 62 are nuclei of slightly older oöcytes with the large masses broken up into smaller ones, and the volume of these intranuclear granules is also apparently increased. Fig. 63 is a tangential section of a somewhat later stage showing the beginning of the network which replaces the masses and granules in older oöcytes. These granules, as well as the reticular network, stain like the chromosomes with iron-hæmatoxylin, borax carmine, saffranin, and Benda's stain for mitochondria. Figs. 64 and 65 show the characteristic network which lines the nuclear membrane of older oöcytes of *Sagitta bipunctata*. The pattern consists of two parts, the network and the irregular figures in the openings. These two parts vary in prominence in different species. In *Sagitta bipunctata* the meshwork is more prominent, and in older eggs the central figures disappear first. Figs. 66 *a* and *b* are tangential and median sections of the same nucleus, Figs. 67 *a* and *b* similar sections of the nucleus of an older egg, showing the breaking up and gradual absorption of the network as the egg approaches maturity. When the chromosomes have been reduced to the maturation size, the stainable network has practically all disappeared from the nuclear membrane (Fig. 68). In *Sagitta minima*, one finds in young oöcytes stellate or amœba-like figures (Figs. 69 and 70), which later unite to form a network (Fig. 71), which may or may not have rather indistinct figures in the spaces (Fig. 72). In *Sagitta inflata* I usually find such a pattern as in Fig. 73, and Fig. 74 from an older egg. In these three species the network is more prominent than the contained figures. In *Sagitta elegans* it is the reverse—the central figures

are heavier and more lasting than the surrounding network (Fig. 75). Fig. 76 is from an older egg, where the network has nearly all disappeared and the central figures are spread out thinner. In *Sagitta decipiens* (Fig. 77 and 78) we find the irregular figures without the network.

As the above described intranuclear network is evidently derived from the large chromatin-like masses and granules of the younger oöcytes, and these masses and granules are either developed under the influence of the chromosomes, or, more probably, are material extruded from the chromosomes of the early-growth stage, I think we must regard it as comparable to the chromatin-like material frequently given off from the chromosomes in early growth stages of both spermatocytes and oöcytes (see Boring, '07, Figs. 62-67, and King, '08, Figs. 26-30), and not to the mitochondria which is found outside the nucleus, usually in the form of fibers, and which has not been satisfactorily traced to a nuclear origin. Ordinarily when one sees such material thrown out by the chromosomes or spireme, one is inclined to regard it as waste material, but the case of *Sagitta*, where the material forms such a conspicuous pattern on the nuclear membrane during the greater part of the growth stage of the oöcyte, strengthens the growing opinion that such material may have a specific function in connection with the growth-process of the egg. So far as I can see, there is no evidence whatever that either the chromatin-like masses seen against the nuclear membrane in spermatogonia, oögonia, and young oöcytes, or the elaborate pattern of older oöcytes, can possibly be derived from Elpatiewsky's 'besondere Körper' or from the degenerate inner connecting, or fertilization cell of the ovum, as Buchner ('10) has suggested.

#### SYNAPSIS AND SPERMATOGENESIS

In regard to synapsis, I have no new evidence to add to that given in my two earlier papers. I have found nothing opposed to the conclusion that in *Sagitta* we have a case of parasynapsis in the oöcyte and telosynapsis in the spermatocyte. Synizesis and bouquet stages (Figs. 79-81) are not uncommon among the

young oöcytes, but it is very difficult to find the synapsis stages figured in my '05 paper, Pl. 16, Figs. 18–25. The chromosomes are extremely small and favorable stages are rarely met with.

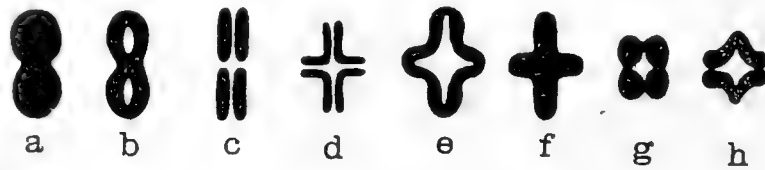
In my first paper on *Sagitta* ('03) I briefly described the principal stages in the spermatogenesis of *Sagitta bipunctata*, showing the reduced number of chromosomes to be nine, formed by telosynapsis. Mention was made of the fact that one or two large nucleoli were often seen near one or both poles of the first maturation spindle. In a later paper ('05) on the spermatogenesis of insects, some additional figures (Pl. 7, Figs. 226–241), based on further study of the body spoken of as a nucleolus in the earlier paper, were given. This was of especial interest at that time on account of its possible homology with the heterochromosomes of insects.

After further study of the element in question in *Sagitta elegans*, new material of *Sagitta bipunctata* from Pt. Erin and Helgoland, and considerable work on *Sagitta bipunctata*, *S. minima*, and *S. inflata*, in aceto-carminic preparations, at Naples last year, I am convinced that the elements figured as  $x$  in the spermatogonia, and growth stages of the spermatocytes (Pl. 7, Figs. 226–233) are nucleoli comparable to those described above in oögonia, oöcytes and spermatogonia, while the elements so designated ( $x$ ) in the maturation mitoses (Figs. 235–241) are products of premature division of one of nine bivalent chromosomes. I find some individuals in which the nine chromosomes all behave alike in all or in most cases; while in other individuals, one divides prematurely and appears at or near the two poles of nearly every spindle in both first and second spermatocytes. In the first material, which I prepared at Naples and studied at Würzburg in 1902, I found only a very few cases of this phenomenon; so few that I did not publish any figures. The figures in the 1905 paper were taken from material obtained from Naples the same year, but used at the time only for the study of the maturation stages of the ova. *Sagitta elegans* showed the same peculiarity, and the appearance of daughter chromosomes at each pole of the maturation spindle in metaphase was much more frequent. In the Pt. Erin material the difference between individuals in this

respect was very striking. In some specimens all of the chromosomes were in the equatorial plate without exception; in others the polar chromosomes were seen in every spindle; in still others both conditions were found in the same cyst. This variation was also found in the aceto-carminic preparations of the three Naples species. The number of chromosomes in all of the species studied is the same, nine for the reduced number. The individual chromosomes are much more distinct in aceto-carminic preparations, and I think there can now be no doubt as to the count.

Figs. 82 and 83 are first spermatocyte equatorial plates of *Sagitta bipunctata* from spindles in which all of the chromosomes were in the equatorial plates; Fig. 84 from a spindle where eight were in the equatorial plate and one daughter chromosome ( $x_1$  and  $x_2$ ) at each pole. All of the spindles from this individual showed the polar chromosomes. Some of these spindles also showed the tetrad nature of the chromosomes (Fig. 85). Fig. 86 is a prophase showing the premature division of one chromosome ( $x$ ). *Sagitta inflata* proved to be the best material for studying the spermatocytes with aceto-carminic. Fig. 87 is an equatorial plate containing the nine chromosomes, and Fig. 88 a side view of a spindle with the two daughter chromosomes ( $x$ ) near the poles of the spindle and in different positions, so that they appear somewhat unequal in size. In spindles which do not show the prematurely divided chromosome, one often sees a larger bent chromosome (Fig. 89), which apparently is the erratic one, and may or may not divide precociously. It will also be noticed that this chromosome is attached to the spindle fibers in a different way from the others. Fig. 90 shows the polar chromosomes in such a position that they look equal, and in Fig. 91 they are already dividing precociously a second time. Fig. 92 is a second spermatocyte spindle showing the polar chromosomes. Fig. I, *a* to *h*, shows the various forms which the bivalents may assume in first spermatocyte prophases and metaphases. The dumb-bell form (*a*) is by far the most common; *h* I have seen only once; while the other figures are not infrequent in aceto-carminic preparations. Fig. 93 also shows in an oblique view of a daughter plate the splitting of the chromosomes for the second maturation mitosis.

I have as yet been able to find no satisfactory explanation of the fact that in the four species in which the spermatogenesis has been examined, one of the nine chromosomes behaves so peculiarly, sometimes dividing synchronously with the others, sometimes precociously in both maturation mitoses. One naturally wonders if this chromosome is in any way homologous with the heterochromosomes of insects, whose distribution is closely connected with the determination of sex. Of course in a hermaphrodite organism, there is no question about determination of sex, as the two sexes are combined in one individual, and all fertilized eggs must contain both male and female sex determiners, or at least must be able to produce both male and female germ cells.



# I

Fig. I. Forms which the bivalent chromosomes assume in prophases and metaphases of the first spermatocyte.

So far as the chromosomes are concerned, there is no evidence of any such reducing mitosis as might give oögonia, oöcytes and ova purely female, and spermatogonia, spermatocytes, and spermatozoa purely male. If sex determiners are present in the germ cells, both must be present in both oöcytes and spermatocytes and reduction must give both eggs and sperm of two kinds with reference to sex. Selective fertilization would then be necessary to give eggs containing both determiners, and capable of producing hermaphrodite organisms. It may then be possible that the 'besondere Körper' discovered by Elpatiewsky is a mechanism for determining dominance of the sex determiners in the male and female germ cells; *i.e.*, determining that one pair of primary germ cells shall give rise to ovaries, the other pair to testes. In support of this suggestion it is important to determine whether anything comparable to this 'besondere Körper' is present in the

segmenting eggs of other hermaphrodite organisms, and in either male or female germ cells of those insects in which an equal pair of heterochromosomes has been described in the male germ cells.

It is my intention to investigate further some of the points referred to in this paper as soon as there is opportunity to work again on fresh material.

I desire in this place to express my appreciation of the opportunity afforded me to collect material at Pt. Erin, Helgoland and Naples, and of the privileges and courtesies which I enjoyed at the Zoologisches Institut, Würzburg, and at the Stazione Zoologica, Naples.

#### SUMMARY

1. Eggs of *Sagitta*, when ripe, apparently make their way into a previously closed oviduct and move down the oviduct to the reproductive pore by their own activity.

2. The ovary proper and the ducts are entirely distinct structures as to their origin. Each ovary develops from one of the four primary germ cells, while the antrum and oviduct develop from a fold or outgrowth of the mesodermal layer of the body wall below the ovary, and the cells which form the sperm-duct or 'spermentasche' originate from the oviduct wall by migration or delamination.

3. It is suggested that the deeply stained network that can often be seen in the median part of the oviduct wall next to the sperm-duct may be of an elastic nature.

4. In *Sagitta elegans* eggs free in the ovary have been found in all stages between that of the metaphase of the first maturation mitosis and a 16-cell stage. No satisfactory explanation of this phenomena was apparent.

5. Elpatiewsky's 'besondere Körper' was found in all of these free eggs, beginning with the stage in which the two pronuclei were in the center of the egg.

6. No connection between this 'besondere Körper' and the secondary accessory fertilization cell could be traced.

7. The two accessory cells with the fertilization canal are found in all of the species studied (5), and have been observed in living specimens.

8. The author confirms Elpatiewsky's conclusions as to the 'besondere Körper' in the primary germ cells, and also as to the probability that the division which produces two germ cells is the differential mitosis.

9. The granules and network found on the inside of the nuclear membrane of immature oöcytes appear not to be mitochondria, but material derived from the chromosomes of the very young oöcytes. Whether this is waste material or material which functions in connection with the growth of the oöcytes is an open question.

10. All of the species examined have nine chromosomes in the spermatocytes, one of which sometimes behaves like an equal heterochromosome bivalent. The precocious division of this chromosome is very striking in some individuals and entirely absent in others.

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## EXPLANATION OF FIGURES

(Figures reduced  $\frac{1}{3}$  from the camera drawings)

Figs. 1 and 2. Longitudinal optical section of male (*t*) and female (*o*) primary germ cells in *Sagitta inflata*,  $7\frac{1}{2}$  days. 2mm.-8.

Fig. 3. Cross-section of  $7\frac{1}{2}$  day *Sagitta* through one of the primary oögonia (*o*). 1.5 mm.-12.

Fig. 4. Section through young ovary containing 6-7 oögonia. 2 mm.-8.

Figs. 5 and 6. Sections through two ovaries of the same individual containing 16-20 oögonia. 2 mm. 6.

Fig. 7. Section next below the ovary of Fig. 6, showing beginning of antrum and oviduct (*od*). 2 mm.-6.

Figs. 8 and 11. Sections of same ovary. 2 mm.-6.

Fig. 8. Posterior section of ovary proper showing only one oögonium (*o*).

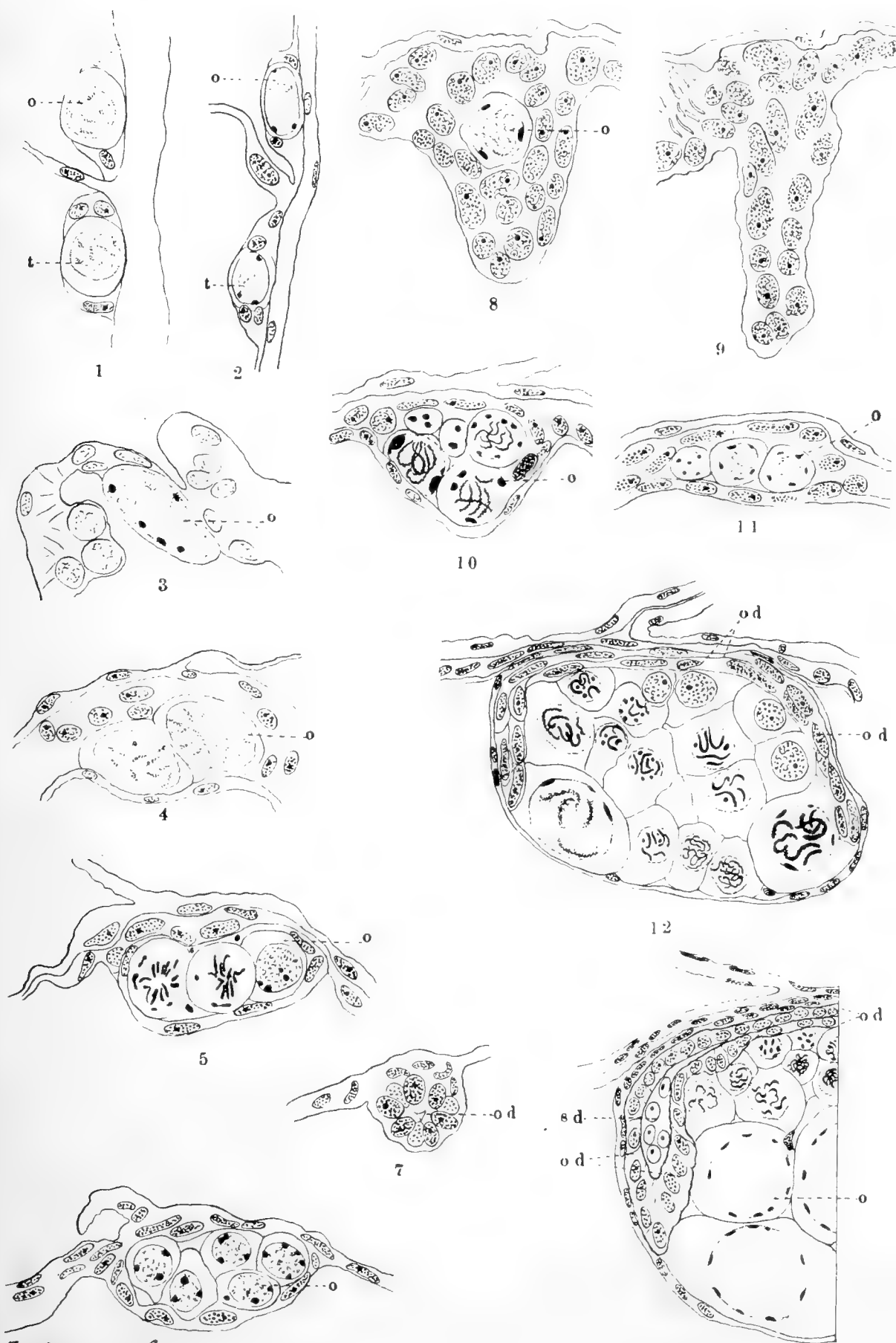
Fig. 9. Next section posterior to Fig. 8, showing the fold or outgrowth of mesoderm which will give rise to antrum and oviduct.

Fig. 10. Fifth section anterior to Fig. 8.

Fig. 11. Tenth section anterior to Fig. 10.

Fig. 12. Cross-section of older ovary, showing the oviduct (*od*) as a 2-layered crescent-shaped structure. 2 mm.-4.

Fig. 13. One-half section of older ovary showing sperm-duct tissue (*sd*) in one wing of the oviduct (*od*). 2 mm.-4.



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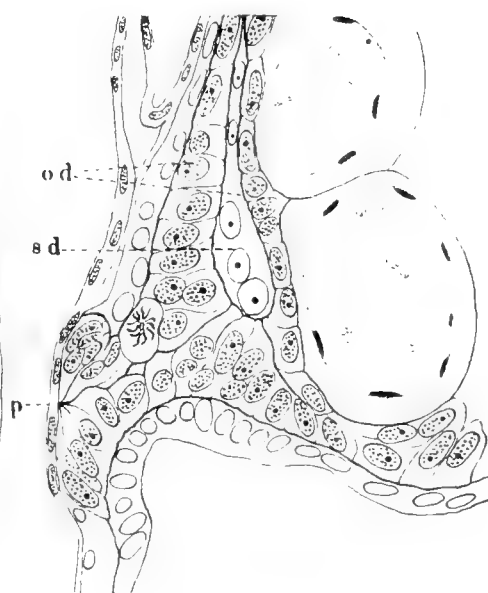
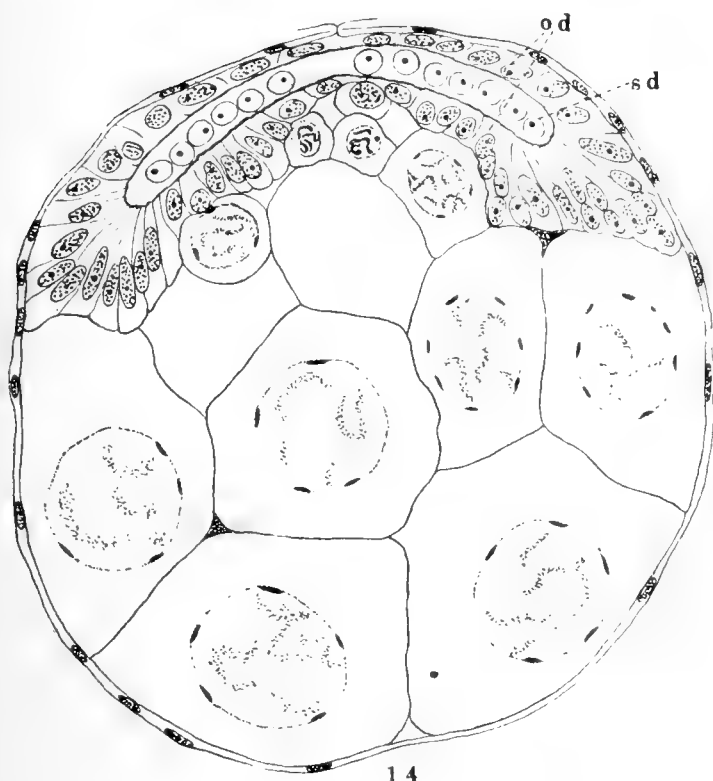
Fig. 14. Older ovary, showing sperm-duct tissue (*sd*). 2 mm.-4.

Fig. 15. Longitudinal section through the antrum region before the external opening (*p*) has become functional. Same stage as Fig. 14. 2 mm.-4.

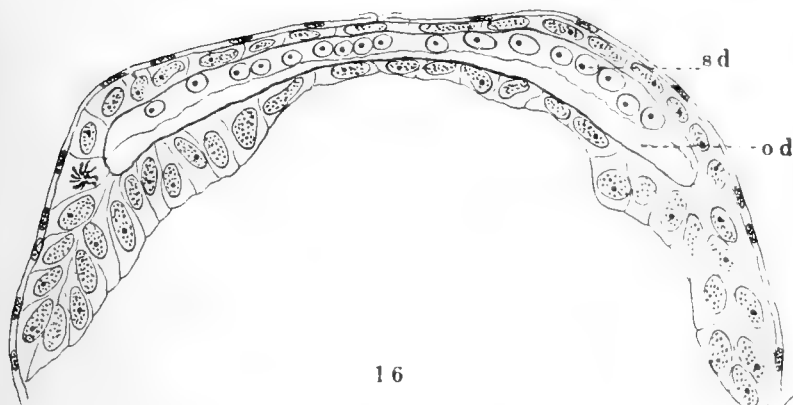
Fig. 16. Crescent-shaped oviduct, showing an opening (*od*) between the sperm-duct tissue (*sd*) and the median wall. 2mm.-4.

Fig. 17. Oviduct and sperm-duct contracted laterally. 2 mm.-4.

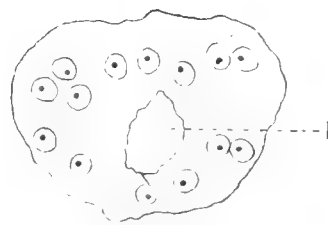
Figs. 18-20. Sperm-duct, showing development of lumen (*l*). 2 mm.-4.



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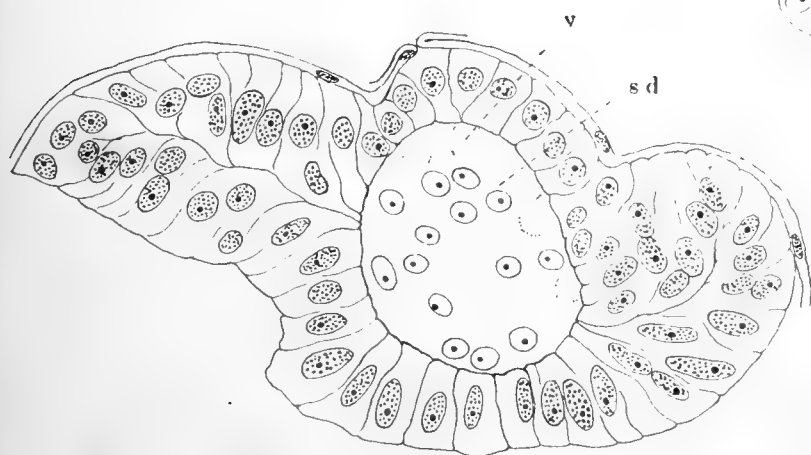
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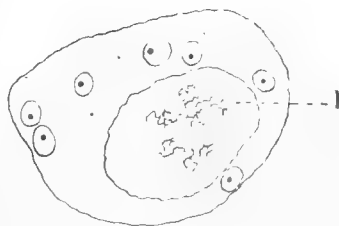
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Fig. 21. Longitudinal section of adult, showing sperm-duct (*sd*) slightly open to the exterior. 2 mm.-4.

Fig. 22. Cross-section of adult ovary, showing an egg (*o*) in the oviduct (*od*). B. and L.  $\frac{1}{5}$ -A.

Fig. 23. First polar body and second polar spindle of an egg free in the ovary. 2 mm.-12.

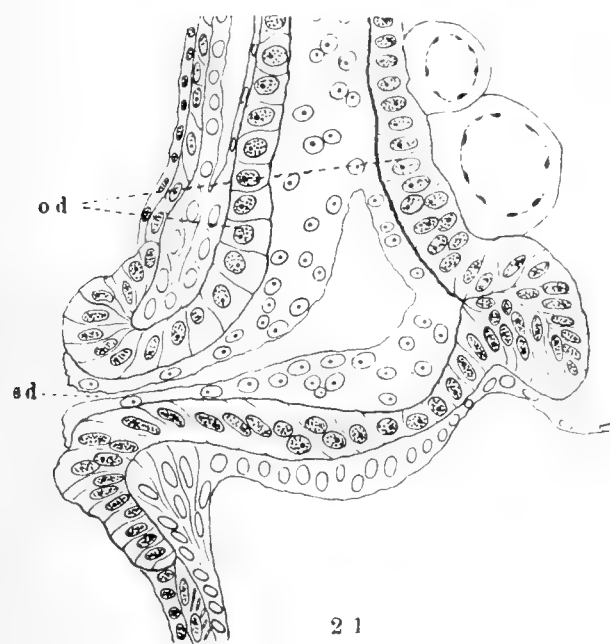
Fig. 24. Two pronuclei of egg free in the ovary. D-4.

Fig. 25. First segmentation spindle of free egg. 2 mm.-4.

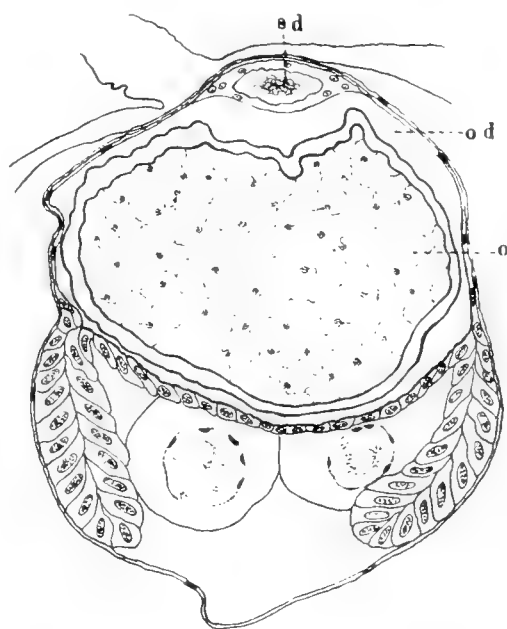
Fig. 26. Second seg. spindle of free egg. 2 mm.-4.

Fig. 27. Section of egg in 8-cell stage, free in the ovary. D-4.

Fig. 28. 'Besondere Körper' of same egg as Fig. 24. 1.5 mm.-4.



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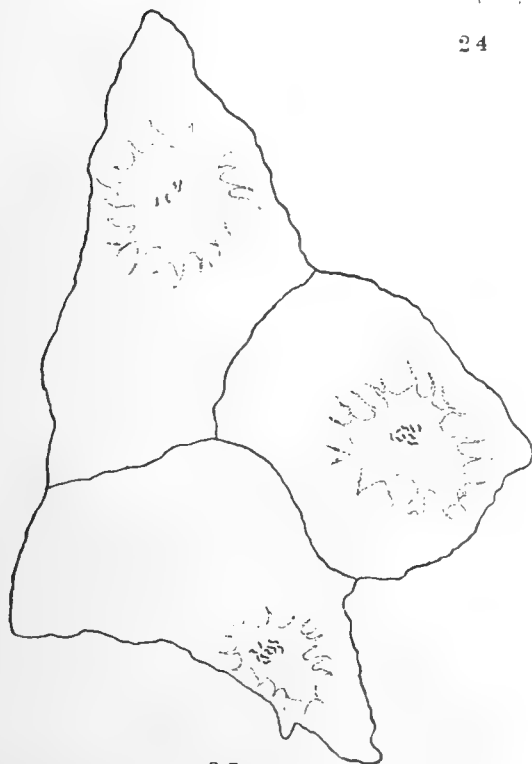
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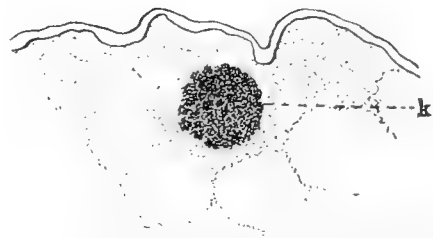
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Figs. 29 *a* and *b*. Free egg containing 'besondere Körper' (*K*) and pronuclei (*n*). 4 mm.-2.

Fig. 30. Two-cell stage of egg free in the ovary, showing the 'besondere Körper.' 4 mm.-2.

Fig. 31. Section of 16-cell stage, free in the ovary. 4 mm.-2.

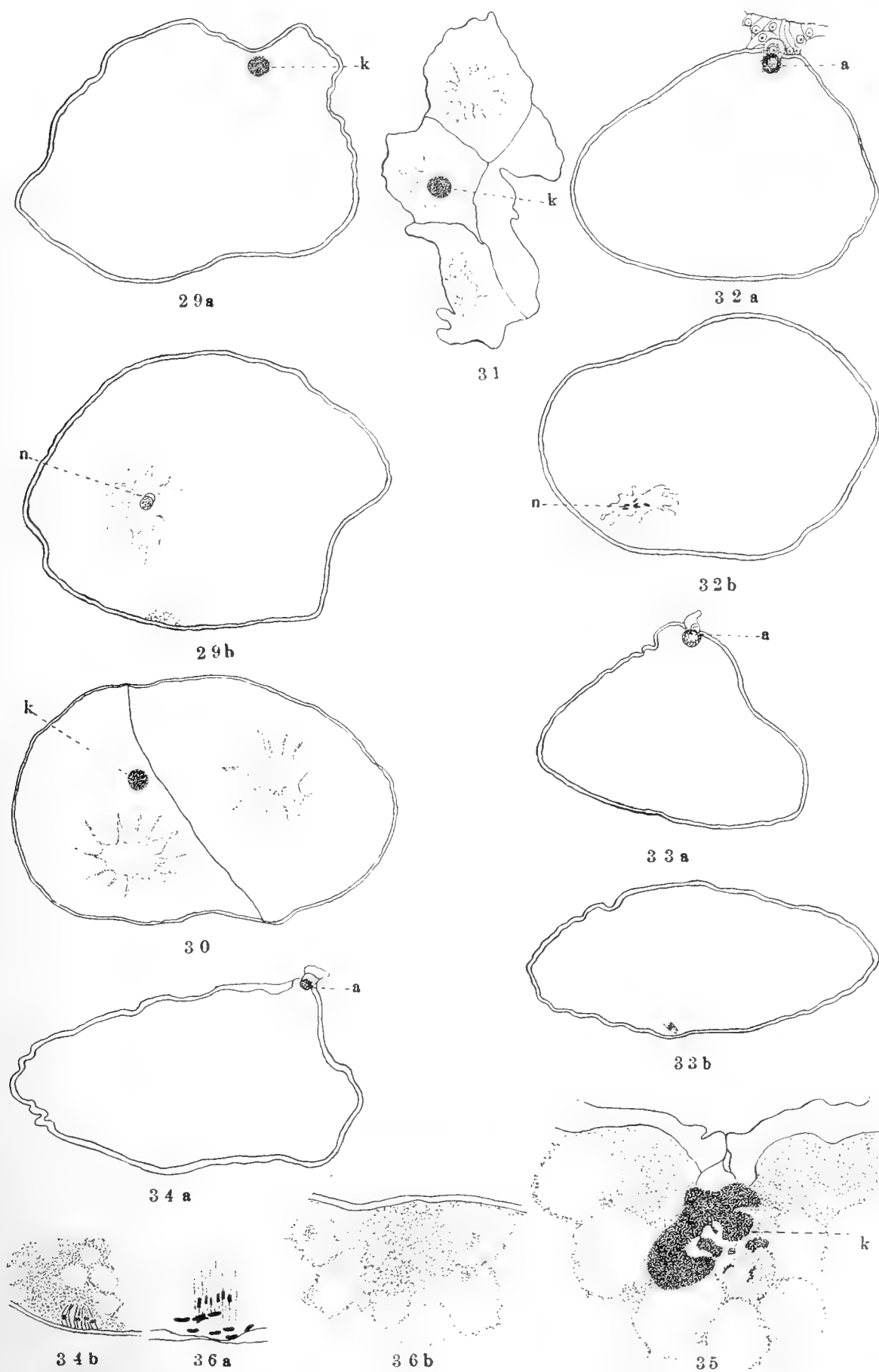
Figs. 32 *a* and *b*. Sections of attached egg, showing degenerating accessory cell (*a*) and nucleus (*n*) breaking down for maturation. 4 mm.-2.

Figs. 33 *a* and *b*. A slightly later stage, egg still attached but mat. spindle formed. 4 mm.-2.

Figs. 34 *a* and *b*. Slightly later stage. 4 mm.-2.

Fig. 35. Exceptional case where the 'besondere Körper' (*K*) is irregular in form. 1.5-6.

Figs. 36 *a* and *b*. Second mat. spindle (*a*) and spot of denser cytoplasm at vegetal pole (*b*) of free egg. 1.5 mm.-2.



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Fig. 37. Nucleus of egg near maturity, showing cast-out granules of chromatin-like material.

Fig. 38. Section through the two accessory fertilization cells ( $a_1$   $a_2$ ), showing granular body ( $b$ ) and spermatozoon ( $s$ ) in the canal. 1.5 mm.-4.

Fig. 39. Optical section of accessory cells in living egg of *Sagitta inflata*, showing canal ( $c$ ) and granular body ( $b$ ). 2 mm.-4.

Fig. 40. Section showing spermatozoon in second accessory cell. 1.5 mm.-4.

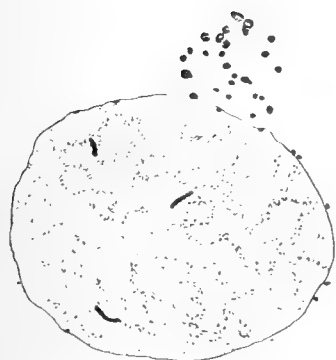
Figs. 41 *a* and *b*. Two sections through the accessory cells of an egg of *S. minima*. 1.5 mm.-4.

Fig. 42. Similar section from *S. elegans*. 1.5 mm.-4.

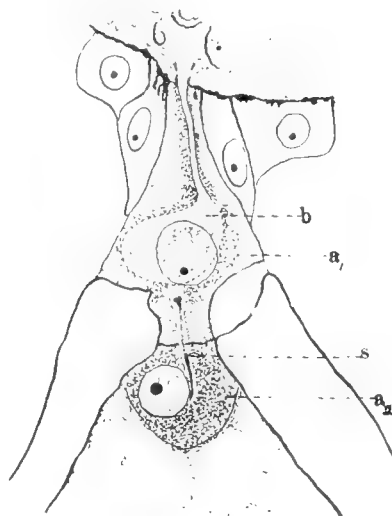
Fig. 43. Sections from *S. elegans*, showing degeneration where an egg has broken away without entering the oviduct. 1.5 mm.-4.

Fig. 44. Section of the accessory cells of a young egg of *S. inflata*. 1.5 mm.-4.

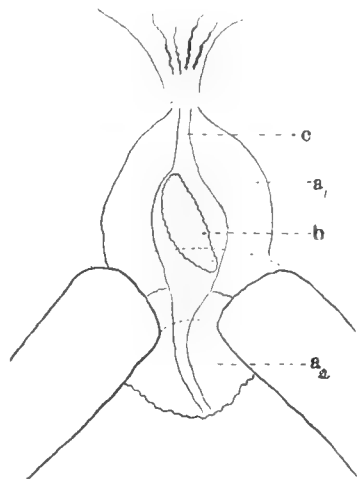
Fig. 45. Similar section of an older egg. 1 mm.-4.



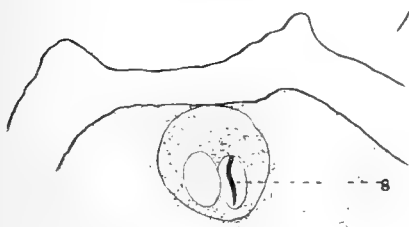
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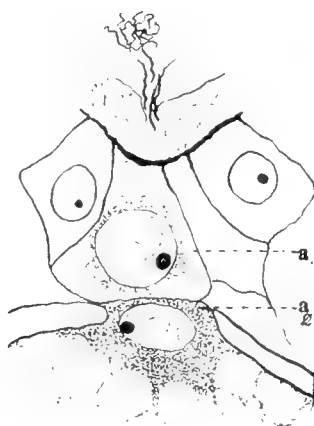
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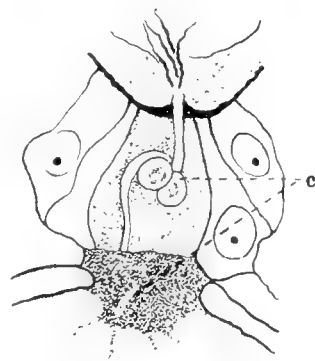
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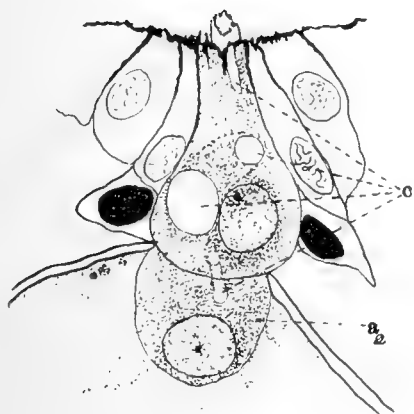
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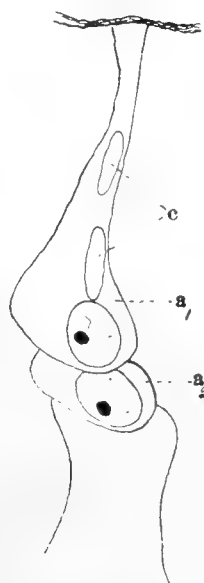
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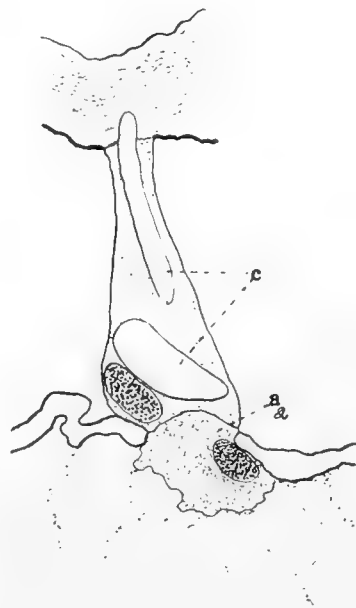
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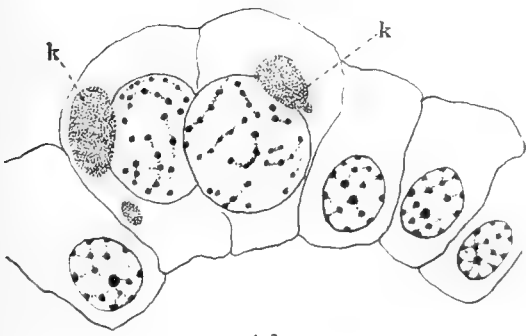
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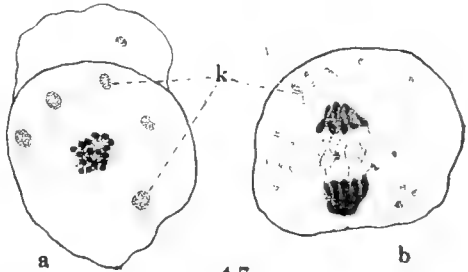
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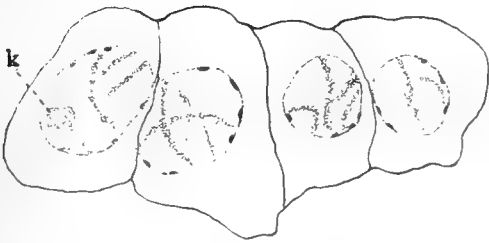
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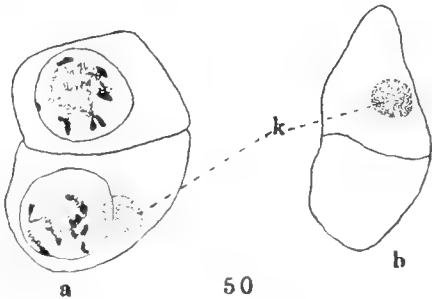
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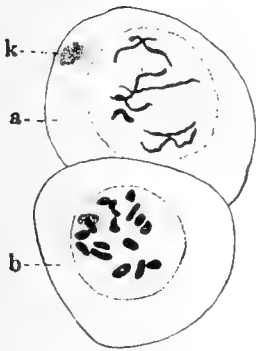
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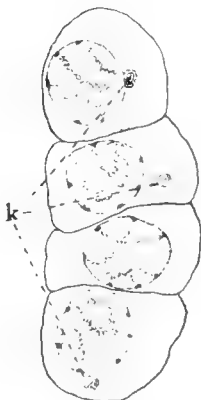
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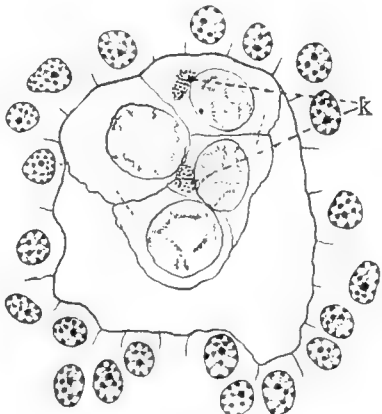
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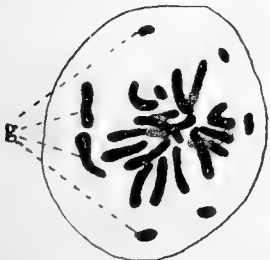
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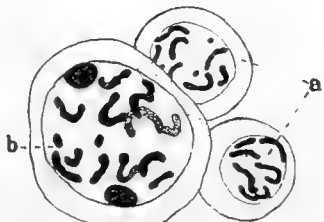
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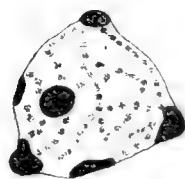
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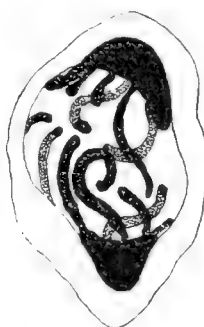
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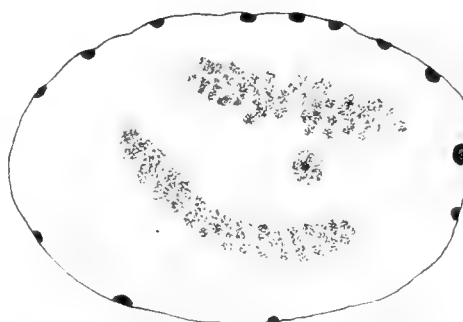
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66 a



66 b



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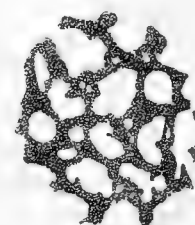
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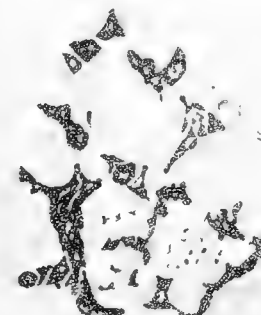
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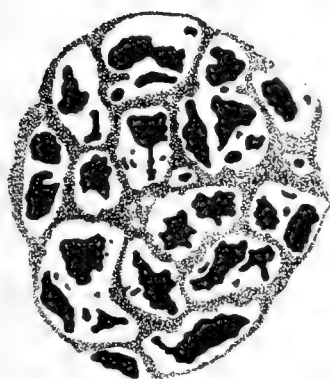
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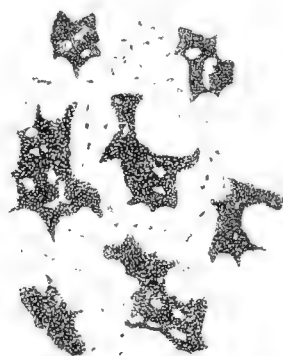
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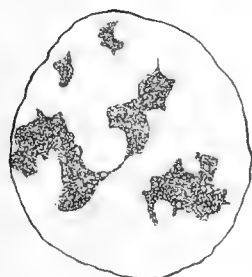
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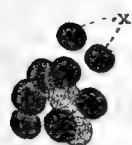
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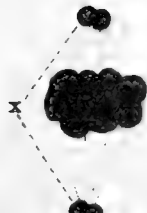
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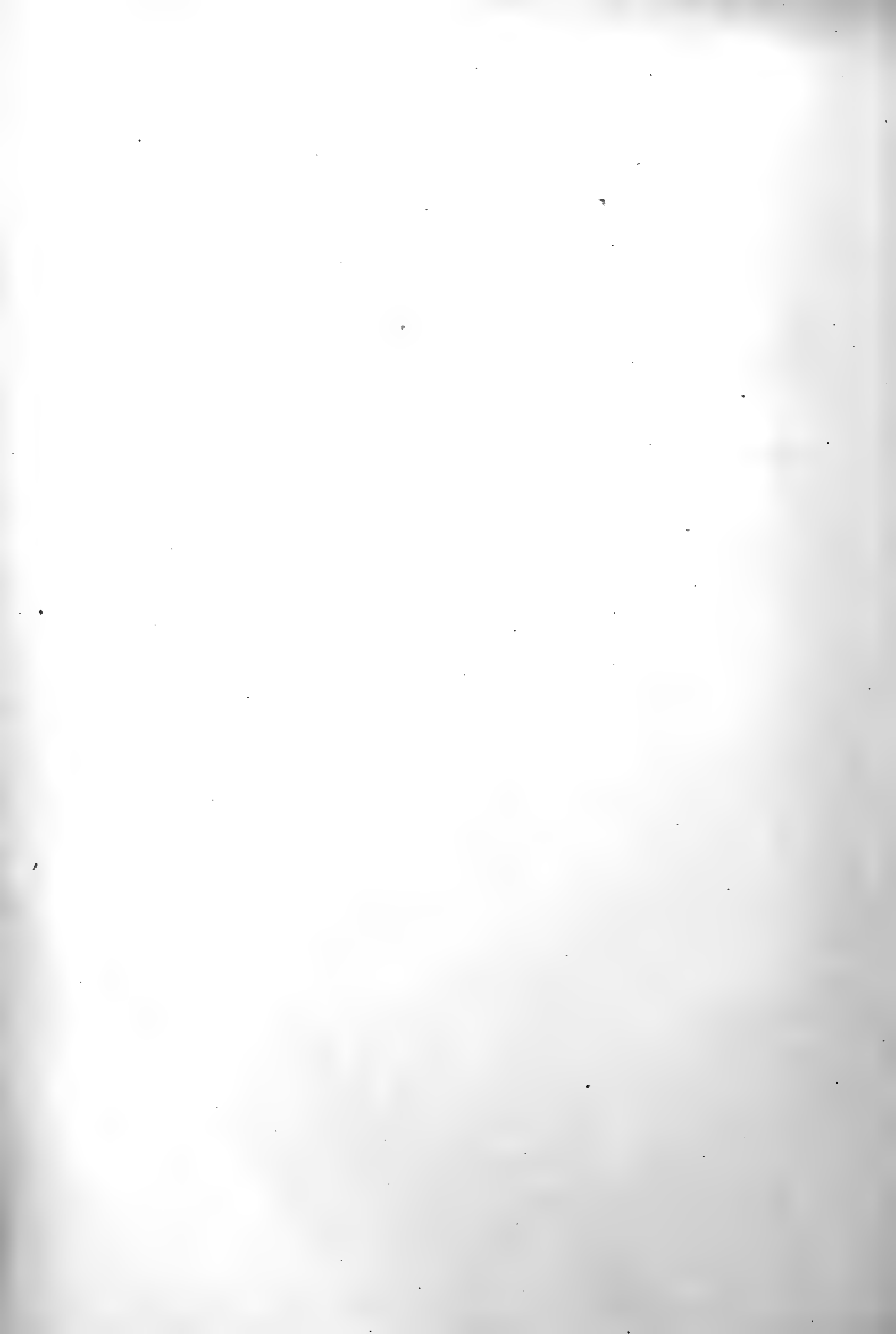
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# THE MORPHOLOGY OF THE PINEAL REGION IN TELEOSTS.

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*From the Department of Comparative Anatomy, Harvard Medical School*

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## INTRODUCTION

In 1901 Minot published an account of the morphology of the pineal region in which it was shown that the characteristic structures included therein had their beginnings in constant well-defined divisions of the fore-brain roof. These subdivisions constitute a succession of arches and intervening projections into the ventricle, and in *Acanthias* are recognizable in embryos of 11.5 mm.

in length, a stage in which the diencephalon and telencephalon are demarcated. The names proposed for them, taken in order from before backward, are:

- |                      |                          |
|----------------------|--------------------------|
| 1. Paraphysal arch   | 4. Superior commissure   |
| 2. Velum transversum | 5. Epiphysis             |
| 3. Post-velar arch   | 6. Posterior commissure. |

According to the author, "The homologues of all these parts, exist probably in all vertebrates."

Other questions suggested and discussed in the paper concern the distinction between the paraphysis and paraphyseal arch, the genesis of the choroid plexus, the significance of the great difference of development of the post-velar arch among animals, the relations of the superior commissure and the position of the posterior commissure with reference to the diencephalon and mesencephalon.

Following the direction of research given in Minot's paper, Dexter ('02) and Warren ('05), in investigations of the avian, amphibian and reptilian brains, have supported the view of the general occurrence of the primitive subdivisions and also have noted especially the distinction between the paraphyseal arch and organ.

The present study<sup>1</sup> was undertaken with the object of extending the same line of inquiry to the teleostean brain. In view of the differences in the mode of formation of the medullary tube in the elasmobranchs and teleosts, misgivings were felt at the start that the early form of the fore-brain roof would necessitate an interpretation of the value of its parts in terms of the elasmobranch type rather than present parts easily identified and directly comparable with the divisions of the shark's brain. To some extent this was true, but the early appearance of the epiphysis, posterior commissure and velum was sufficient to make certain the interpretation of the remaining regions. The method of study adopted

<sup>1</sup> This investigation was made during the year 1906-7 while the writer held an Austin Teaching Fellowship in the Department of Histology and Embryology in the Harvard Medical School. For the opportunities there afforded for anatomical research and especially for the encouragement received from the director of the department, Professor Minot, he is deeply grateful.

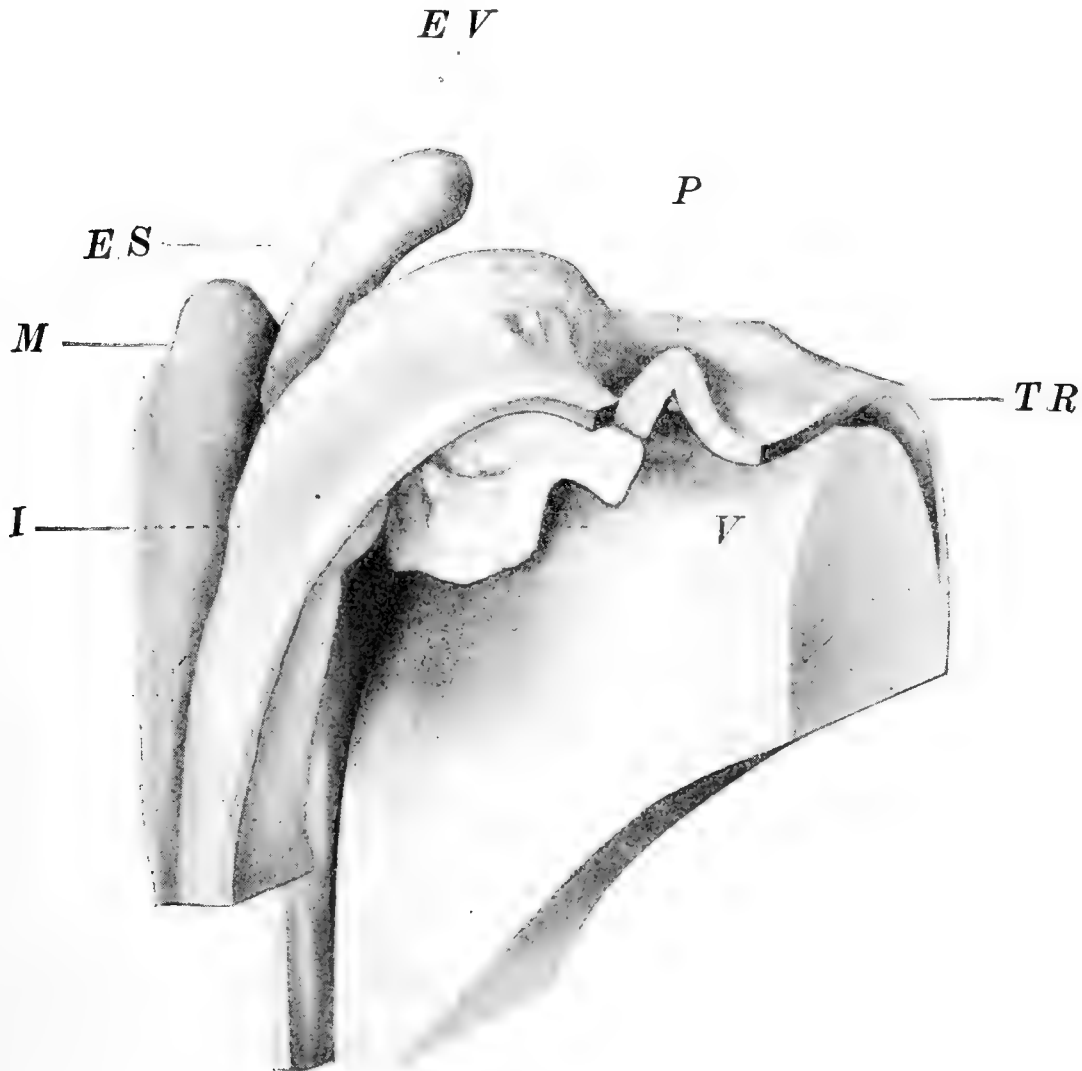


Fig. 1. Reconstruction of the pineal region of an *Opsanus* embryo of 11 mm. H.E.C., series no. 121.  $\times 167$ . A part of the right wall of the ventricle has been removed and the paraphysis has been sectioned in the median plane.

has also helped in the identification of the regions in the smallest embryos. As the starting point, there was selected from the series an embryo in which all the structures peculiar to the pineal region could be identified beyond question. Each structure was then traced back through smaller and smaller embryos to the stage in which it was first apparent.

The material basis for most of the observations recorded is *Opsanus* (*Batrachus*) *tau*, the toad-fish, a teleost represented in

the Harvard Embryological Collection by an extensive series of sections of embryonic and larval stages. In addition, the brains of specimens half and fully grown were obtained and prepared for dissection and microscopical study. Other fishes included in the research are *Salvelinus*, *Fundulus*, *Ameiurus*, *Lepidosteus*, *Amia*, *Acanthias* and *Petromyzon*.

## I DESCRIPTION

### *Opsanus* Embryos of 11 mm. in Length

*Epiphysis.* In embryos of this stage the epiphysis is located in the middle of a depression intervening dorsally between the mid-brain and telencephalon (fig. 1, *ES*). Its direction is nearly dorsad, corresponding in this respect with the posterior epiphysis of *Salmo* embryos of 7 mm. Hill ('94) found the posterior epiphysis in larval salmon of 13 mm. directed forward, so that it presented dorsal and ventral surfaces. The end of the organ lay close to the epidermis, whereas in *Opsanus* it reaches a level half-way between the diencephalic roof and the ectoderm. Differentiation of the outward form has already begun and sagittal sections (fig. 2, *E*) show an ovoid end-vesicle surmounting a cylindrical stalk. The latter expands at its base where it joins the diencephalic roof, and presents a slight curve in the sagittal plane in adaptation to the superior commissure lying just anterior. The epiphysis of *Opsanus* at the present stage, like that of *Clupea* (Holt '91), is a solid structure. These teleosts differ, therefore, from the embryos of *Salmo* in which, as shown by Hill, the epiphysis is hollow. Sections of the solid epiphysis give evidence, however, of a difference in the structure of the peripheral and central regions, the former appearing deeply, the latter but lightly stained. Moreover the protoplasm of the peripheral coat is nucleated, whereas that of the central region is non-nucleated.<sup>2</sup> This sort of structure is

<sup>2</sup> There are no cell boundaries in the protoplasm of the epiphysis at this stage; the organ has a syncytial structure and the nuclei are all located in the peripheral parts, forming a more or less even layer.

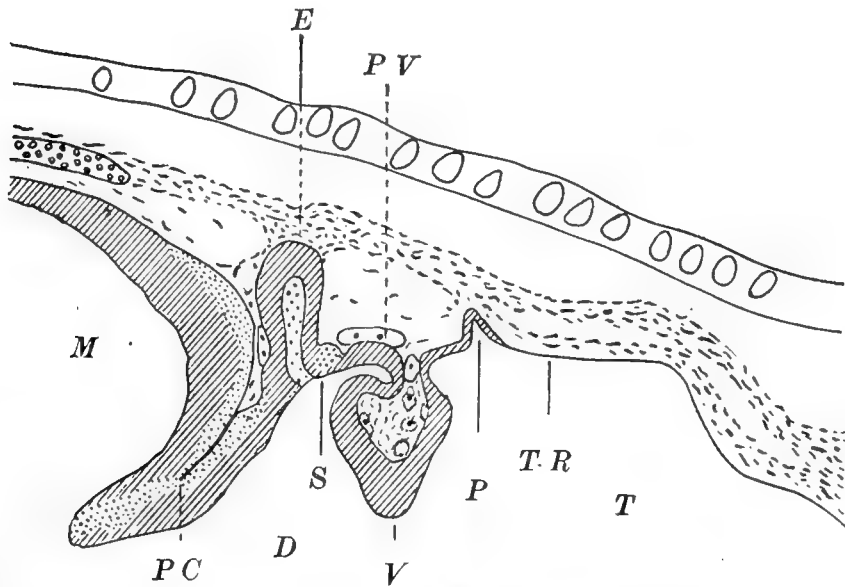


Fig. 2. Median section of the pineal region of an *Opsanus* embryo of 11 mm. H.E.C., series no. 122, section no. 118.  $\times 130$ .

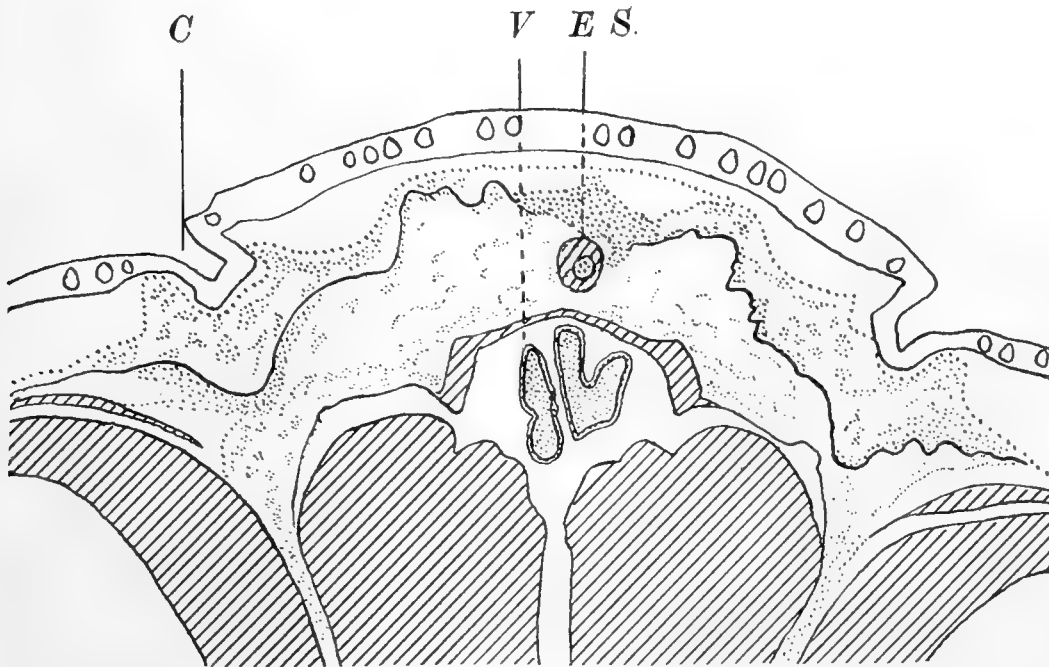


Fig. 3. Transverse section of the pineal region of an *Opsanus* embryo of 11 mm. H.E.C., series no. 121, section no. 99.  $\times 97$ .

to be seen in both the end-vesicle and stalk, the clear central portion of the latter extending quite to the base and to the margin of the ventricle. In later stages a cavity forms, taking the place of the region of lightly-staining protoplasm, and the peripheral nucleated layer then becomes a surrounding wall. The thickness of the peripheral layer varies somewhat in different parts of the vesicle, but there is no marked contrast in this respect between the anterior and posterior regions of the layer. In transverse (fig. 3, ES) and in frontal sections, the epiphysis is seen to have a median position. The point of union of the stalk with the roof of the third ventricle is also midway between the intermediate tubercles; that is to say, there is no approximation of one or the other of the tubercles toward the middle line. Regarding the presence of a second epiphyseal organ, the following observation was made. In the series of transverse sections no. 121, a small rounded body was found to the left of the median line anterior to the epiphysis and surmounting the superior commissure (fig. 4, A). In section no. 101 (fig. 5, A) continuity was traced between this body and the roof of the third ventricle anterior to the commissure. Structurally it consists of a peripheral nucleated stratum surrounding a central clear area of protoplasm.

The depression on the dorsal aspect of the brain between the mesencephalon and telencephalon is filled with a loose mesenchymal network. This tissue surrounds the epiphysis and connects the end-vesicle with the membranous roof of the cranium by a dense broad band. Hill states that the end of the epiphysis in *Salmo* of 13 mm. projects into a mass of undifferentiated tissue lying between it and the epidermis. Blood vessels are present at the sides and at the back of the epiphyseal stalk. One of them is constant and traverses the mesenchyma in the median plane. The pigmentation of the tissues about the epiphysis described by Cattie ('82) appears in the later stages of development of *Opsanus*. Differentiation of the epidermis over the pineal region does not occur except in connection with the formation of the pit organs of the lateral line system.

*Superior Commissure.* Fig. 2, taken from a median sagittal section of the pineal region of an *Opsanus* embryo of 11 mm.,

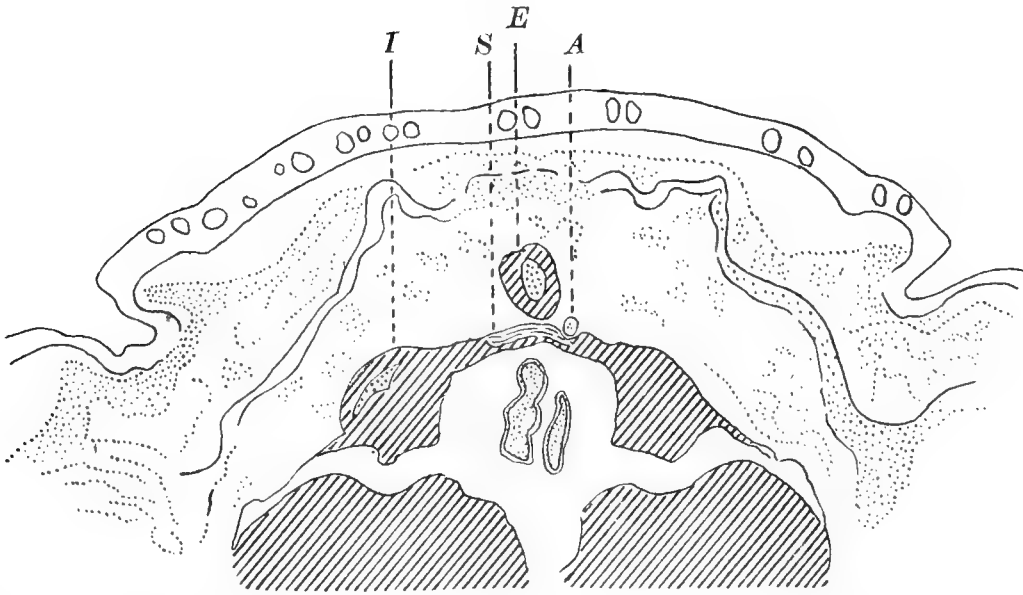


Fig. 4. Section no. 102 of the same series as fig. 3.  $\times 97$ .

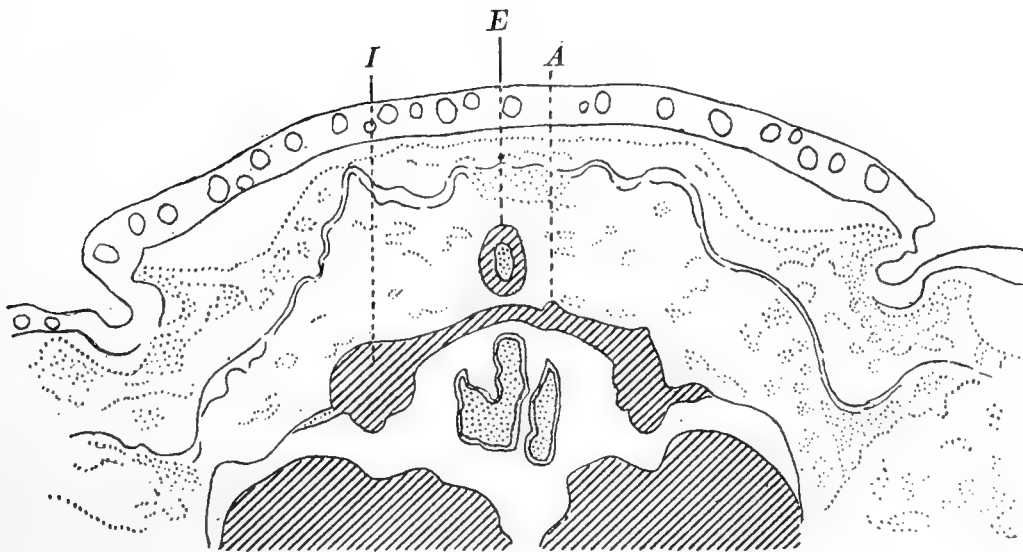


Fig. 5. Section no. 101 of the same series as fig. 3.  $\times 97$ .



shows the superior commissure (S) lying immediately anterior to and in contact with the base of the epiphysis. It occupies a position in the outer part of the diencephalic roof, resting ventrally upon a thin ependymal layer (Comp. Acanthias, 22 mm., Minot, '01). The component fibers, non-medullated at this period, pass over the surface of the post-velar arch to the intermediate tubercles.

*Posterior Commissure.* The posterior commissure is located in that fold of the brain-roof which is recognized as the boundary between the mid-brain and fore-brain. Sagittal sections show it to be folded transversely. The two layers resulting from this disposition of the commissure are continuous at their ventral edges. They extend from side to side and lie, the one dorso-caudad of the other (fig. 2, PC). Rabl-Rückhard ('82), Haller ('98), Mayser ('82), and other writers, have noted these two divisions in teleosts. Haller has described the commissure in *Salmo* as composed of a dorsal and a ventral part, the former belonging exclusively to the lobi optici and carrying fibers from lobe to lobe, the latter made up of mixed fibers of the longitudinal tracts. In the toad-fish, a septum of mesenchyma, continuous with the same tissue around the epiphysis and mid-brain, separates the two layers for a considerable distance. The ventral or anterior stratum, an even layer of fibers, is separated from the third ventricle by a thick ependyma. It reaches forward as far as the epiphysis, occupying that region of the diencephalic roof called the Schaltstück or pars intercalaris (Burckhardt). Some of the fibers pass around the base of the epiphyseal stalk. The dorsal or posterior stratum is related to the wall of the mid-brain. Its thickness varies inversely with that of the underlying epithelium in such manner that, whereas the ectal surface of the stratum is even, the deep surface is irregular. When followed in the dorsal direction, this stratum merges with the ectoglia layer of the mid-brain roof.

*Velum transversum.* (Fig. 1, V). This structure, projecting from the roof of the brain into the ventricle some distance in front of the superior commissure, is naturally divisible into two parts. One of these is a low, broad, transverse fold of the roof, the other a large ovoid body, hanging from the middle of the fold by a short pedicle (fig. 2, V). In the embryos of teleosts, ganoids, and elas-

mobranchs, the velum consists of a simple reduplication of the epithelium of the brain-roof. Such a velum is found in *Opsanus* at an earlier period of development and persists as the transverse fold of the present stage (fig. 6, VL). The median lobe, although constituting the greater part of the velum at this time, is a secondary modification of the middle part of the fold. The latter, followed anteriorly, goes over into the paraphysis, posteriorly into the post-velar arch. On either side, a prominent sagittal ridge of the fore-brain roof can be seen extending backward within the

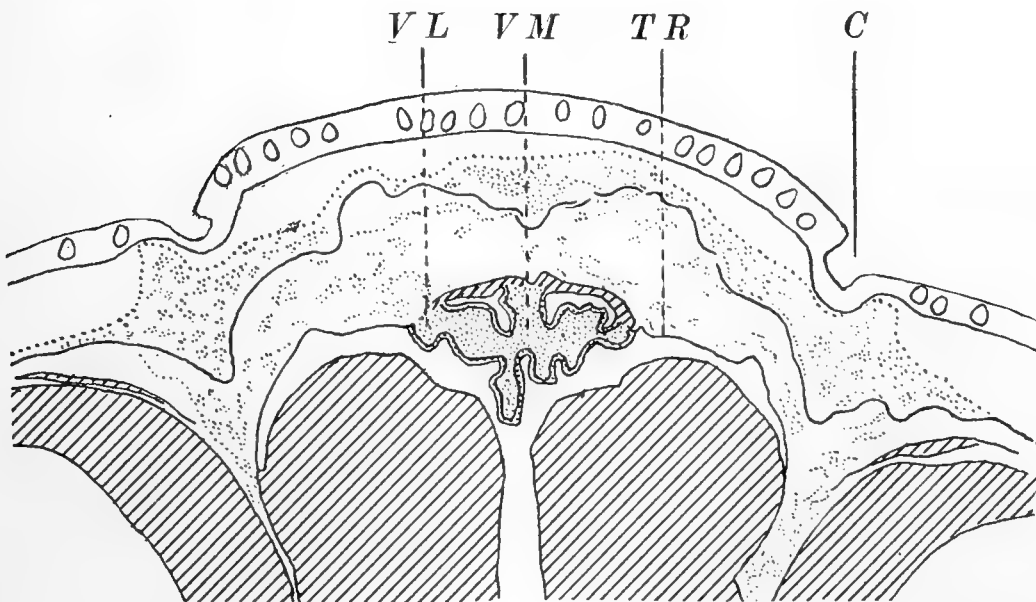


Fig. 6. Section no. 97 of the same series as fig. 3.  $\times 97$ .

ventricle to the intermediate tubercle (figs. 3, 5). It marks the lateral extent both of the velum and paraphysis. Regarding the contour of the median lobe, one finds the surface broken by fissures of greater or less depth, dividing the whole mass into lobules. The part of the lobe lying behind the level of the pedicle is greater than that anterior. It will be seen by referring to fig. 2, that the lobe projects some distance caudad beneath the post-velar arch. The velum, even at this early stage, affords evidence of being adapted to a secretory function. Sections of the lobe show a peripheral thick epithelial tunic, thrown into folds and sup-

ported by mesenchyma rich in blood-vessels. The latter, continuous with vascular channels in the vicinity of the epiphysis, post-velar arch and paraphysis, are close under the velar epithelium. No difference was observed in the thickness of the epithelial coat of the anterior and posterior aspects of the velum as has been described in *Acanthias* by Minot ('01) and in *Acipenser* by Kupffer ('06).

*Post-velar Arch* (Fig. 2, PV). The name "post-velar arch" was given by Minot ('01) to the curve in the brain roof which lies between the velum and the epiphyseal anlage. This is the part of the diencephalic roof called *Zirbelpolster* by Burckhardt and the region of the post-paraphysis of Sorensen ('94). Out of this region is developed in the ganoids and some of the bony fishes the evagination called by Goronowitsch ('88) the dorsal sac. In *Opsanus*, the arch does not expand into a sac, but on the contrary diminishes in extent and finally disappears. In the stage under discussion, the post-velar arch is a small dome-shaped evagination of the diencephalic roof extending sagittally between the superior commissure and velum and going over laterally into the intermediate tubercles. At no time is the evagination so extensive as it is in *Salmo* where, as Hill ('94) states (p. 242) "it bears some resemblance in form to the epiphysis." The arch in *Opsanus* rises a little higher than the base of the epiphysis but not so high as the paraphyseal fold. Its simple cavity can be followed forward to the pedicle of the velum, on either side of which it becomes continuous with a short blind recess (fig. 6). In transverse sections through the posterior part of the velum, these two small recesses appear as lacunae surrounded by the velar and ependymal epithelium. In structure the post-velar arch consists of a layer of rather thick ependyma. Anteriorly, this continues with slight increase in the height of its constituent cells into the velar epithelium. Posteriorly, it changes abruptly to a very thin layer at the level of the superior commissure. The latter lies upon the epithelium of the caudal part of the arch, over which it extends in a lateral direction as far as the intermediate tubercles. Folds of the epithelium are not present, nor are there any diverticula such as Herrick ('91) has found in the walls of the dorsal sac of

Lepidosteus. In a word, there is no differentiation of this part of the diencephalic roof; it resembles the simple post-velar arch, found by Minot in *Acanthias* embryos. The vascularity of the dorsal sac and of the region around it was observed by Balfour ('77) and has been described by subsequent investigators. Blood sinuses of considerable size are present in *Opsanus* in the mesenchyma dorsad of the post-velar arch, and are connected with the vessels of the epiphysis and velum.

*Paraphysis.* (Fig. 2, P). Gaupp's ('97) and Studnička's ('05) reviews of the extensive literature of the pineal region contain few references to the paraphysis in teleosts. The question of its presence in the class has received little attention. Three years after Selenka's ('90) discovery of the organ, Burckhardt found a paraphyseal rudiment in the trout. Later, Studnička ('95) described a paraphysis in two teleosts, *Lophius* and *Anguilla*. In adult *Lophius* the paraphysis appears as an evagination of the brain wall in front of the velum. Its occurrence is not constant. In young *Anguilla* the organ is a relatively large, thin-walled sac connected by a narrow base with the brain. In 1905 the same investigator described the paraphysis in two other bony fishes, *Cepola rubescens*, in which it appears as a conical sac in front of the velum, and *Belone acus*, where a rudimentary paraphysis is indicated by unevenness of the lamina supraneuroporica. The paraphysis of *Opsanus* is a simple transverse fold of the roof of the telencephalon just anterior to the velum (fig. 1, P). Its walls are composed of an epithelium differing markedly in its greater thickness and staining properties from the tela of the fore-brain. In sagittal sections the paraphysis appears tent-shaped, with an anterior oblique and a posterior perpendicular wall going over into the velar fold. Of these two walls the anterior is somewhat thicker. Followed in the lateral direction the organ is clearly defined to the same extent as the velar fold, that is, as far as the sagittal ridge of the fore-brain roof, described on p. 329. The simple cavity of the paraphysis presents no diverticula and communicates freely with the telencephalic ventricle.

*Opsanus Embryos 8 mm. in Length*

*Epiphysis.* The dorso-caudal direction of the epiphysis at this stage brings its posterior surface into contact with the mid-brain (fig. 7, E). The organ is relatively shorter now and in form

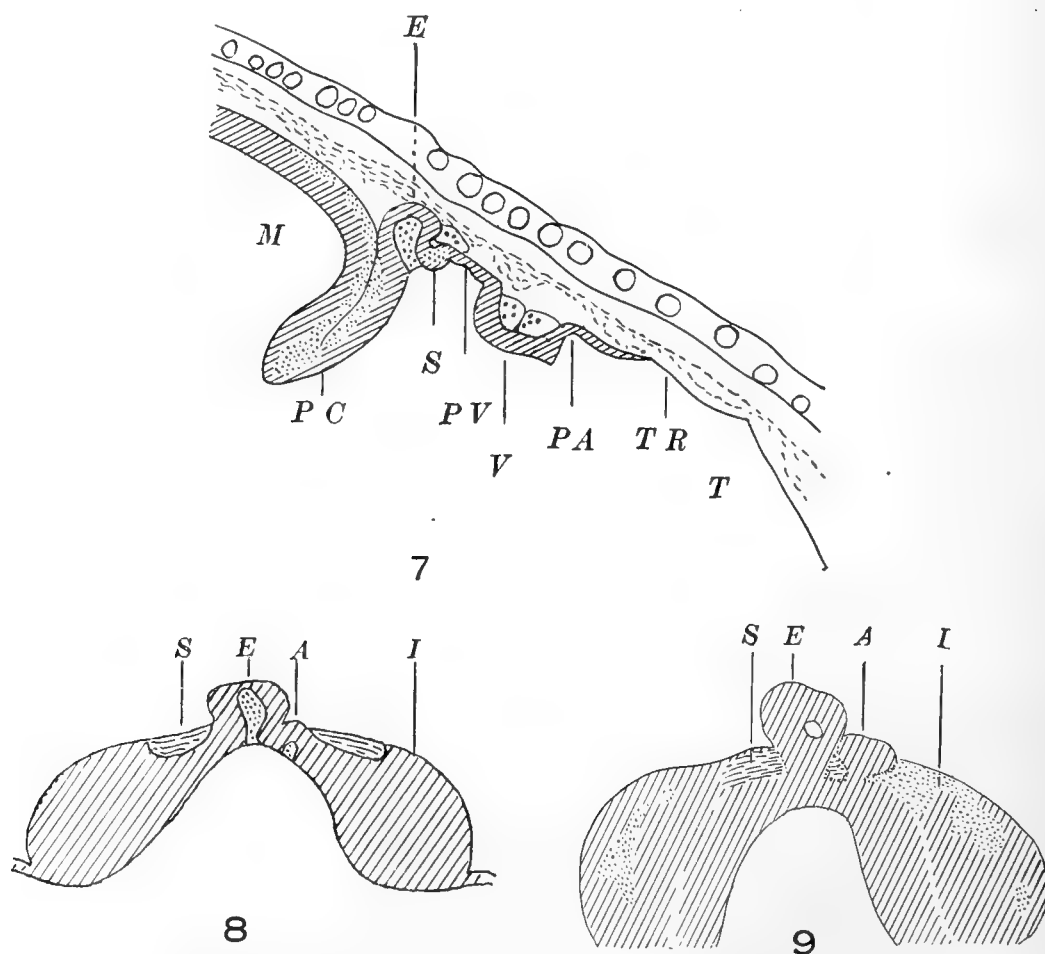


Fig. 7. Median section of the pineal region of an *Opsanus* embryo of 8 mm. H.E.C., series no. 116, section no. 78.  $\times 116.8$ .

Fig. 8. Transverse section of an *Opsanus* embryo of 8 mm. H.E.C., series no. 115, section no. 36.  $\times 116.8$ .

Fig. 9. Transverse section of the pineal region of a *Salvelinus* embryo of 10 mm. H.E.C., series no. 455, section no. 42.  $\times 84$ .

somewhat oval. Its structure is essentially as in the 11 mm. embryo and calls for no particular description. Just to the left of the base of the epiphysis there can be seen in transverse sections of the diencephalic roof an oval, compacted mass of nuclei pro-

jecting above the surface. The center of the mass consists of clear protoplasm, the whole structure bearing a close resemblance to the epiphysis of the 3.5 mm. embryo (fig. 8, A). This small bud is evidently the beginning of the anterior rudimentary epiphysis noted in the 11 mm. embryo. The examination of *Salvelinus* embryos, resulted in the discovery of two epiphyses as was anticipated in view of their presence in *Salmo*. They are represented in transverse section in fig. 9. It will be seen that these bodies are of unequal size, that the larger one is located in the median plane and the smaller one to the left. A cavity is present in the larger epiphysis.

Hill and other investigators have remarked upon the absence of mesodermal tissue between the epiphysis and ectoderm in very young teleost embryos. This condition was observed in the present study in the embryos of *Salvelinus* (fig. 10), *Fundulus*, and also *Amia* (fig. 11). It is not so however in *Opsanus* embryos, for there is always present between the brain and the epidermis a continuous layer of mesenchyma. Within this tissue the end of the epiphysis is to be seen (fig. 7, E). It is worthy of note that, whereas the end vesicle of this organ is pressed closely against the epidermis in the above named fishes, it is farther removed in *Opsanus*. A large blood sinus, lying between the superior commissure and the mesenchymal layer, is in contact with the anterior surface of the epiphysis.

*Superior Commissure.* It is at this stage of development that the superior commissure first appears as a clearly defined bundle of fibers. Its position relative to the epiphysis is the same as in the 11 mm. embryo. Sagittal sections show that the fibers are incompletely separated by a range of nuclei into dorsal and ventral groups.<sup>3</sup> In fig. 7, it will be observed that the commissure lies upon the ependyma of the post-velar arch.

*Posterior Commissure.* Excepting that the fibers of the commissure are spread to a relatively greater extent upon the epi-

<sup>3</sup> Transverse sections reveal an intermingling of certain of the ependymal cells with fibers of the commissure, a condition interesting in connection with Mrs. Gage's ('95) observation that, in *Diemyctylus*, processes of the endymal cells traverse the commissure.

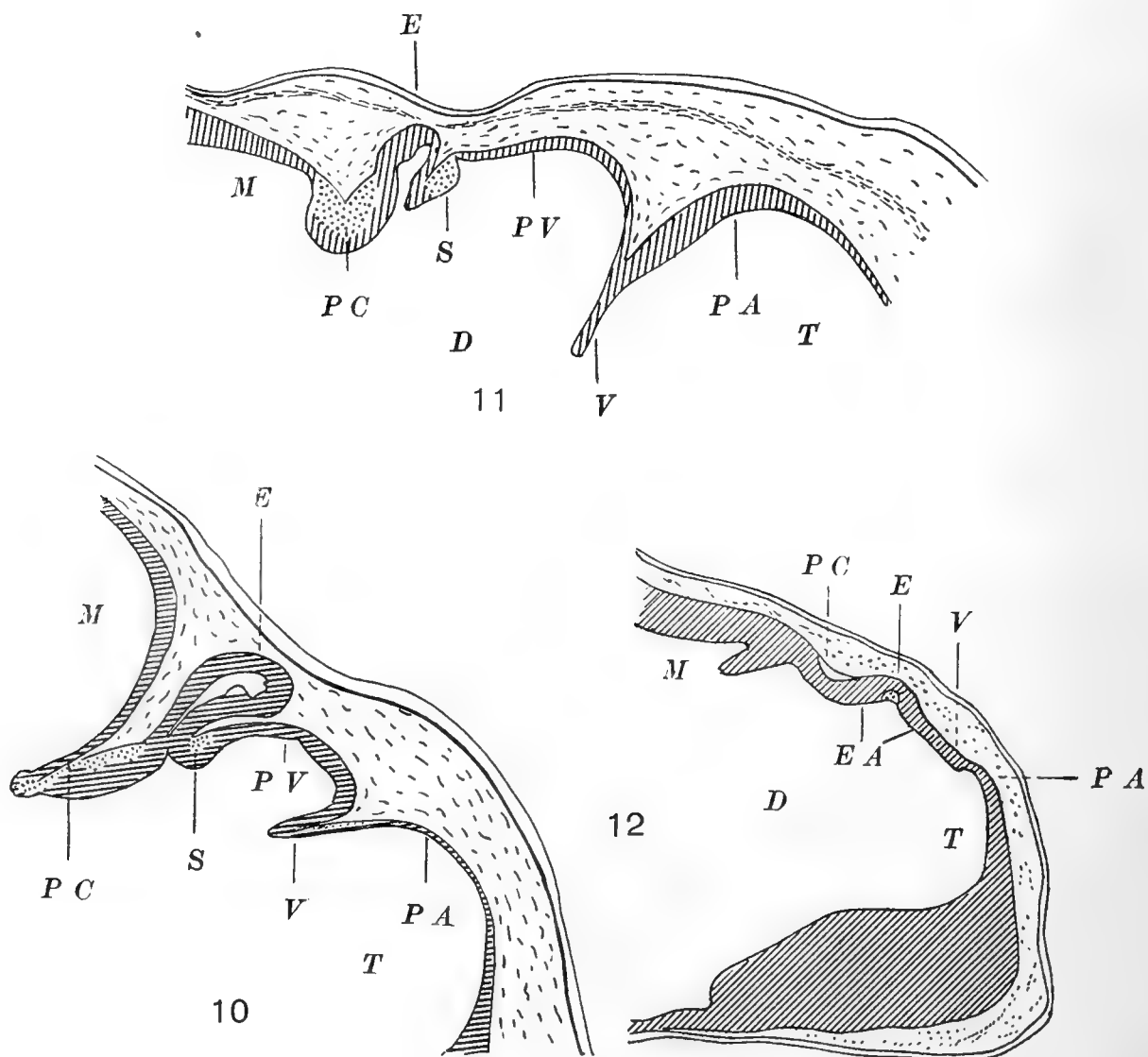


Fig. 10. Median section of the pineal region of a *Salvelinus* embryo of 10 mm. H.E.C., series no. 458, section no. 60.  $\times 84$ .

Fig. 11. Median section of the pineal region of an *Amia* embryo of 10 mm. H.E.C., series no. 12, section no. 91.  $\times 84$ .

Fig. 12. Median section of the fore-brain of an *Opsanus* embryo of 5 mm. H.E.C., series no. 110, section no. 55.  $\times 115$ .

physeal stalk, the conditions described for the 11 mm. stage obtain in the specimens under discussion.

*Post-velar Arch.* Compared with the 11 mm. embryo, the post-velar arch is relatively larger. The evagination is more pronounced and there is presented in consequence a considerable posterior surface over which the superior commissure is spread. This relation is very striking in *Amia* (fig. 11, S).

*Velum Transversum.* The transverse fold and median lobe are both present, the extent and relations of the former being the same as in the 11 mm. embryo. Differentiation in the form of the middle lobe has not begun; its surface is smooth and it is connected directly with the transverse fold.

*Paraphysis.* There is no paraphyseal evagination at this stage. In its place the brain-roof forms a low arch or dome, sharply defined laterally and anteriorly. This paraphyseal arch rises somewhat above the general level of the fore-brain tela, with which it contrasts in its thicker and more deeply staining epithelium. Caudally the arch goes over into the transverse fold of the velum.

*Opsanus Embryos 5 mm. in Length*

*Epiphysis.* Instead of the more or less conical, projecting epiphysis observed in the preceding stages, there is now present a simple, arch-like evagination of the diencephalic roof (fig. 12, EA). The middle of this arch is differentiated both in form and structure from the rest (fig. 12, E). It is raised slightly above the general level and presents in sections the same structural divisions, peripheral and central, as were found in the epiphysis of the older embryos. The arrangement of the nuclei in a superficial layer is interesting in connection with Eycleshymer's ('92) observation of the migration of nuclei in the epiphyseal evagination of *Amblystoma* embryos and of a somewhat similar phenomenon in the optic vesicles. The epiphyseal arch occupies the median plane, its summit lying within the mesenchyma which now fills the interval between the brain-roof and the epidermis. The latter presents no special features in this region. A second epiphyseal outgrowth was not observed and so it appears that this smaller bud is a later development than the principal epiphyseal organ. In *Acanthias*, a projection into the ventricle, seen in sagittal sections, marks the site of the future superior commissure and limits the epiphyseal arch anteriorly. Since this projection is not well defined in *Opsanus*, the arch goes over without sharp limit into the post-velar region.



*Superior Commissure.* This tract is not present.

*Posterior Commissure.* In an embryo of 6.5 mm. the posterior commissure appears in sagittal sections (fig. 13, PC) as a large clear area in the ectoglia of the brain-roof, limited within the deep fold between the fore- and mid-brain. Throughout this area a network of fine processes, continuous with the ependyma, can be seen. The dorsal surface of the commissure presents an indentation, the beginning of the division into the two parts seen in later stages. This indentation is found in *Amia* (fig. 11, P.C) and in the trout as represented in Kupffer's ('06) fig. 150. This investigator says, (p. 131): "Sowohl die Commissura anterior wie die posterior lassen bei der Forelle anfänglich zwei scharf geschiedene Portionen erkennen." In *Opsanus* the anterior part reaches the epiphysis and lies in contact with its posterior surface.

In embryos of 5 mm. the commissure is stretched out in the superficial or ectoglia layer of the brain-roof, caudad of the epiphyseal arch (fig. 12, P C). Haller ('98) has seen a similar disposition in selachians and remarks that it is transitory in the teleosts. The extended posterior commissure has been observed in other forms, as, for example, *Ammocoetes* (See Kupffer, '06, fig. 47), and it has been found in the present investigation in *Fundulus* embryos of 7 mm. The fore-brain in front of the epiphysis presents in sagittal sections an angular bend dividing it into a longer posterior and a shorter anterior segment (fig. 12). Behind the bend a large blood sinus lies in contact with the roof which at this point projects into the ventricle. This is the beginning of the velum transversum. The epithelial roof in front of the angle goes over anteriorly by a slight curve into the thicker terminal wall of the ventricle. This curve is to be compared with Minot's paraphyseal arch.

#### *Opsanus Embryos 3.5 mm. in Length*

Sagittal sections show the brain roof considerably thickened in the epiphyseal region where a slight folding represents the beginning of the epiphyseal arch (fig. 14, EA). A short distance

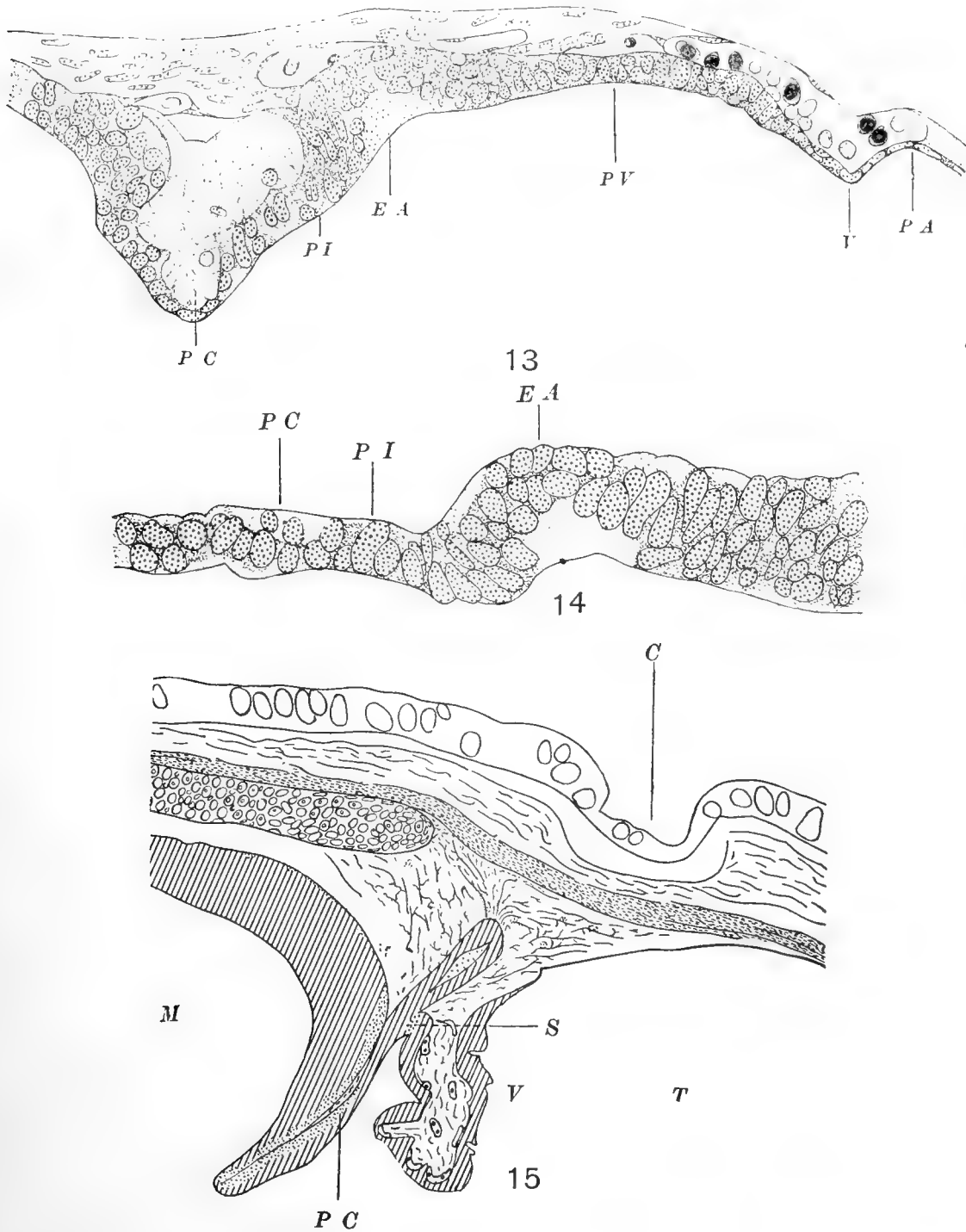


Fig. 13. Median section of the pineal region of *Opsanus*. Length 6.5 mm. H.E.C., series no. 113, section no. 67.  $\times 400$ .

Fig. 14. Median section of the pineal region of an embryo *Opsanus*. Length 3.5 mm. H.E.C., series no. 107, section no. 50.  $\times 500$ .

Fig. 15. Pineal region of an *Opsanus* embryo of 15 mm. Median section. H.E.C., series no. 1183, section no. 145.  $\times 84$ .

caudad, the site of the posterior commissure is indicated by a slight projection of the roof into the ventricle and the presence of a small elongate area of clear protoplasm (Comp. Hill, '99). Between the epiphyseal arch and the site of the posterior commissure, there appears a segment of the brain-roof not seen in the older embryos as a separate region. Its relations identify it as the pars intercalaris (fig. 14, PI). The segment of the roof anterior to the epiphyseal arch is thicker than that which is posterior; also it is relatively and absolutely thicker than the corresponding part in older stages. It presents, as in the 5 mm. embryo, a flexure in the sagittal plane, but exhibits no trace of the subdivisions evident at that stage.

*Opsanus Larvae 15 mm. in Length*

*Epiphysis.* In fig. 15, taken from a section a little to one side of the median plane, the epiphysis is seen to be inclined well forward over the parapyseal region of the fore-brain. The epidermis is now folded to form a deep, wide groove extending transversely between the lines of the supraorbital pit organs (fig. 15C,). Although standing in a position posterior to the level of groove, the epiphysis is inclined forward so that its axis, if prolonged, would meet the groove. This topographical relation is the only one which was observed between the two organs. The small epiphyseal bud, noted in younger stages, is not present. A mesenchymal layer, in which the bony cranial roof is to form, now covers the great dorsal fontanelle of the chondrocranium. Over a considerable area of this layer are attached strands of connective tissue which radiate from the end of the epiphysis.

*Superior Commissure.* Owing to the disappearance of the post-velar arch, the superior commissure now lies between the velum and the epiphyseal stalk.

*Posterior Commissure.* The two divisions are even more sharply limited toward each other than is the case in the earlier stages. The mesenchymal septum is a well defined fold in a membrane which, followed posteriorly, covers the mid-brain, and anteriorly joins with the connective tissue over the diencephalon. The

anterior part of the commissure rests upon the intercalated division of the diencephalic roof, reaching forward to the base of the epiphysis. The posterior division forms a superficial fiber layer of the mid-brain in this region.

*Velum Transversum.* The middle lobe of the velum is relatively larger than in the preceding stage. In its growth backward it has invaded the region of the post-velar arch and has come therefore to lie below the epiphyseal stalk and the intercalated part of the diencephalon. The lobules comprising it are numerous and each includes a blood sinus whose walls are closely related with the epithelial covering of the velum.

*Post-velar Arch.* The reduction of this region, already referred to, goes hand in hand with the backward growth of the velum. The latter appears to have taken up and included the epithelium of the arch. That this process takes place was however not proved, for the epithelium of the arch presents no characters by which it can be distinguished from that of the pedicle and base of the velum.

*Paraphysis.* The thick epithelial coat of the velum passes over into the roof of the telencephalon for a short distance, giving place to the flat epithelium of the tela. Where this change occurs the roof is elevated into a slight but conspicuous transverse fold lying just beneath the end of the epiphysis. This rudimentary paraphysis presents a simple structure, consisting, as in the preceding stages, of a rather high epithelium, resting upon a thin stratum of connective tissue which contains but few vessels.

*Opsanus Larvae of 19 mm. in Length*

*Epiphysis.* The forward inclination of the epiphysis is more marked than in the preceding stage. The elongated stalk, bent over the superior commissure, is continued into the now much enlarged end-vesicle. In the latter a cavity is to be seen for the first time. This space, occupying the region which in earlier stages was characterized by the presence of clear, non-nucleated protoplasm, is traversed by fine fibrillae continuous with the surrounding walls (fig. 16). The conclusion that these fibrils

from a protoplasmic syncytium, derived from the central clear protoplasm and continuous with the walls of the epiphysis rests on the following evidence. The fibrils are first seen at the time of the appearance of the central cavity, not after it is formed. The cavity of the stalk is completed after that of the vesicle and a syncytium of fibrils is seen as the central axis of protoplasm disappears in the process of cavity formation. In staining reactions and in structure the fibrils agree with the central protoplasmic mass, except at the periphery where they resemble, in

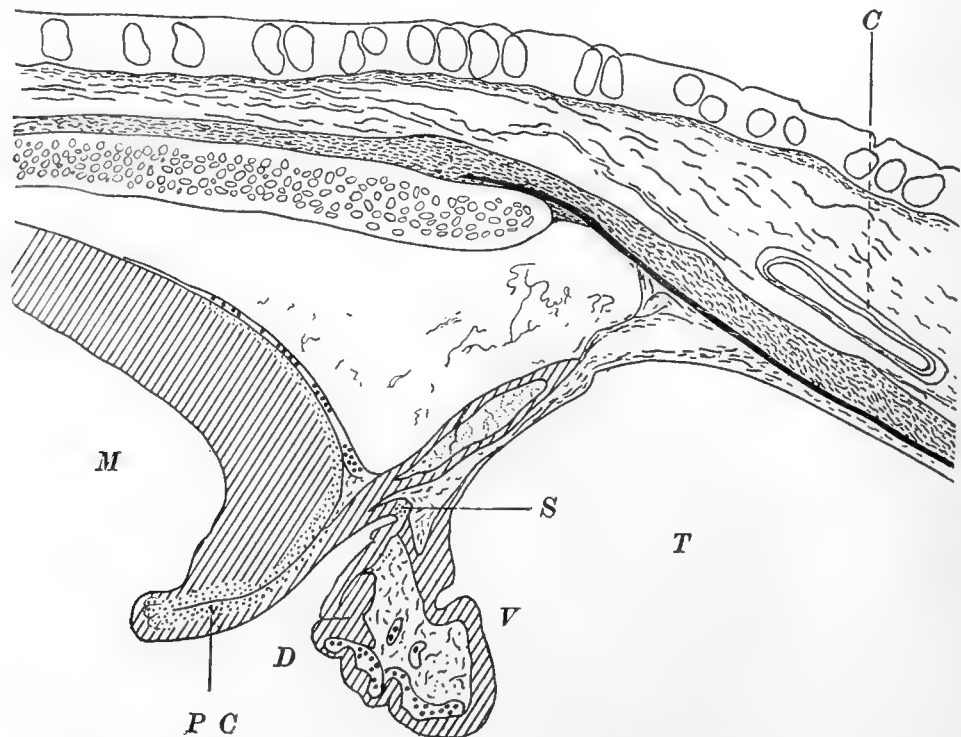


Fig. 16. Pineal region in median section. *Opsanus* of 19 mm. H.E.C., series no. 1188, section no. 203.  $\times 84$ .

these respects, the surrounding walls. There is, therefore, no line of demarcation between the net-work and the protoplasm of the walls. The latter have undergone no differentiation at this time; there are as yet no cell boundaries. Bone has appeared in the cranial roof and to its under side are fixed the connective tissue bundles that radiate from the tip of the epiphysis. A commissural canal has replaced the transverse groove of the integument, seen in the 15 mm. larva, lying somewhat further in advance of the end vesicle of the epiphysis than did the groove (fig. 16 C).



Fig. 17. Dorsal aspect of the brain of adult *Opsanus*.  $\times 7$ .

*Superior Commissure.* This remains as in the preceding stage.

*Posterior Commissure.* The fibers of the commissure are now separated into bundles by delicate septa continuous with the underlying endyma.

*Velum Transversum.* The pedicle of the median lobe now extends backward to the level of the superior commissure. As a consequence of the backward growth of the velum the dorsal part of the diencephalic cavity has been reduced to a mere cleft extending transversely between the intermediate tubercles. The epithelium of the lobules is everywhere elevated into tufts supported by vascular connective tissue.

*Paraphysis.* Velar epithelium extends forward toward the paraphyseal region where there is to be seen a slight transverse fold of the brain-roof. This fold, which appears to be the remains of the paraphysis, forms the anterior end of a median longitudinal groove of the tela, evident in transverse sections. The groove is the beginning of a deep median invagination which in the adult toad-fish separates two diverticula of the caudal end of the telencephalic ventricle.

#### *Adult Opsanus*

*Epiphysis.* The fully developed epiphysis presents a form not uncommon among the teleosts (fig. 17). It consists of an oval end-vesicle terminating a long slender stalk, the whole structure being directed cephalad in the median plane and suspended in the meninges between the fore-brain tela and the cranial roof. The stalk, which is slightly fusiform, measures 6 mm. in length and 0.08 mm. in its greatest diameter. The end-vesicle measures 0.6 mm. in length and 0.38 mm. in greatest breadth. There is no angle between the stalk and vesicle but a gentle curve extends throughout the length of the organ. The cavity, present in both stalk and vesicle, does not communicate with the ventricle. It is traversed by protoplasmic processes forming a wide mesh-work from wall to wall. An artery and vein are associated with the epiphysis throughout its whole extent. In the connective tissue along these vessels and around the distal half of the organ black pigment is present in considerable amount. The commissural canal of the lateral line system is now far anterior to the end of the epiphysis and there seems to be no further relation between these organs. A parietal foramen or fossa of the osseous cranial roof is not present.

*Superior Commissure.* There is no change in the structure and relations of this commissure from what was last observed.

*Posterior Commissure.* The two divisions of the posterior commissure are still recognizable; the intervening connective tissue septum now is less distinct. The anterior division contains some longitudinal fibers which extend in a thin layer around the base of the epiphyseal stalk and on to the intermediate tubercles.

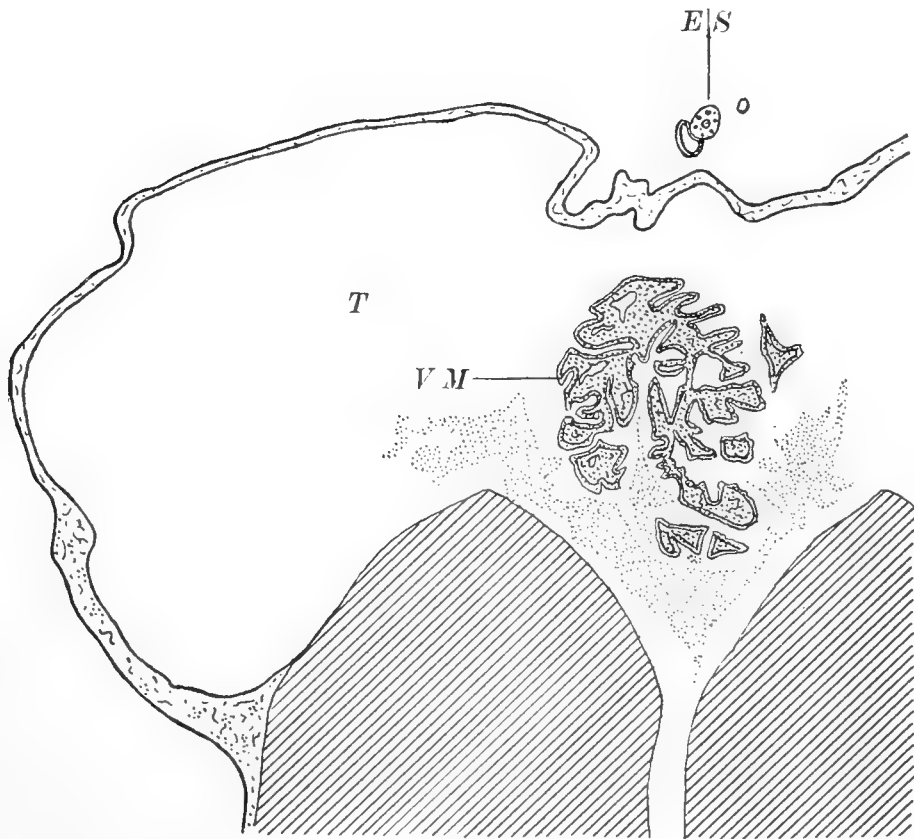


Fig. 18. Transverse section through the pineal region of an adult *Opsanus*. W.U.C., series no. 13, section no. 517.  $\times 21$ .

*Velum Transversum.* The appearance of the median lobe of the velum is much the same as that of the 19 mm. larva. The covering epithelium consists of a single layer of long club-shaped cells, grouped into prominent tufts. The larger free end of the cell is often irregular, fringed or lacerated, sometimes rounded and regular. Neither cilia nor cuticulae are present, but long shreds of some substance extend from the ends of the cells into the ventricle there to become continuous with a coagulum which is always found close about the velum. In the coagulum are rounded bodies and masses which are stained like the clubbed ends of the cells. The nucleus is located near the free end of the cell and, between it and the base, granules are sometimes to be seen which stain deeply. The epithelium rests upon a rather thick reticular membrane, separating it from numerous underlying blood-vessels.



*Post-velar Arch.* This structure can no longer be said to exist, its place being occupied by the velum.

*Paraphysis.* There is no trace of a paraphyseal differentiation of the fore-brain tela. The latter, in its posterior part, has, however, been changed from the simple dome-form of the larval stages by the appearance of bilateral diverticula. In fig. 18, it will be seen that the ventricle is continuous from side to side, but that the tela is depressed in the mid-line to form a broad, shallow groove opposite the stalk of the epiphysis. Fig. 19, which is taken from a section at the level of the velar pedicle, shows a median partition between the two wide diverticula of the fore-brain ventricle. This septum extends from the bottom of the groove, noticed in the previous figure, and contains, besides some large blood-vessels going to and away from the velum, the stalk of the epiphysis.

## II DISCUSSION

### *Morphological Divisions of the Fore-brain Roof*

Burckhardt ('94, a & b) recognized in types of all vertebrate classes the presence and constant relations of the following structures:

Paraphysis	Epiphysis
Velum Transversum	Pars Intercalaris
Zirbelpolster	Posterior Commissure.
Superior Commissure	

The forecast of these structures in the embryonic brain has been described by Minot ('01) who, as mentioned in the beginning of this paper, found that the pineal region of *Acanthias* at an early stage presented six constant divisions. The divisions, in the form of arch-like evaginations and alternating depressions into the ventricle, were named by this author according to their subsequent differentiation. Further observations upon the pineal region of embryos of other animals led to the belief that they were fundamental and that homologous parts might be found in all vertebrates.

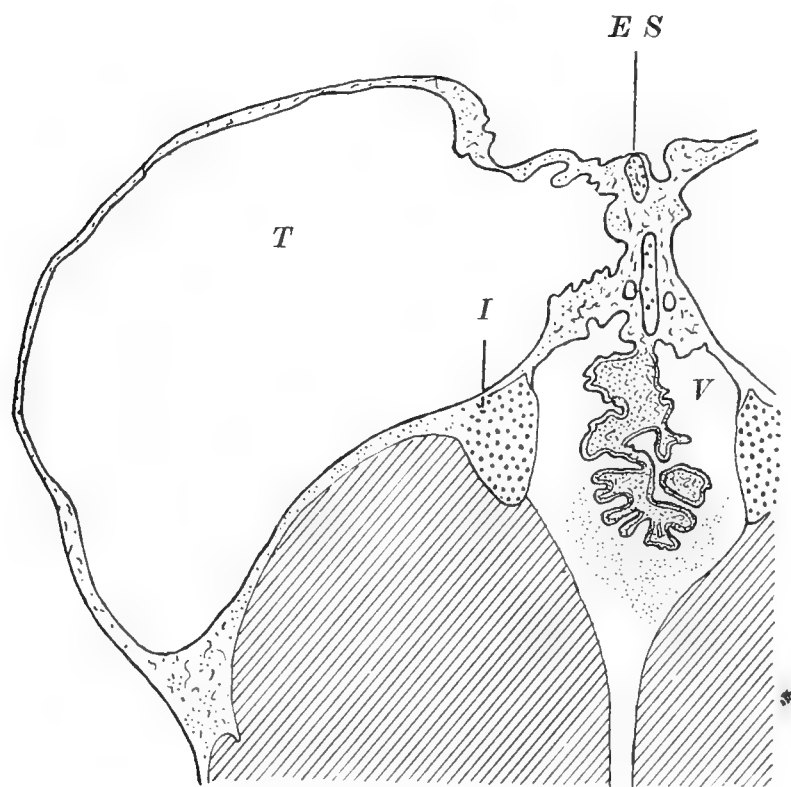


Fig. 19. Section no. 542 of the same series as in figure 18.  $\times 21$ .

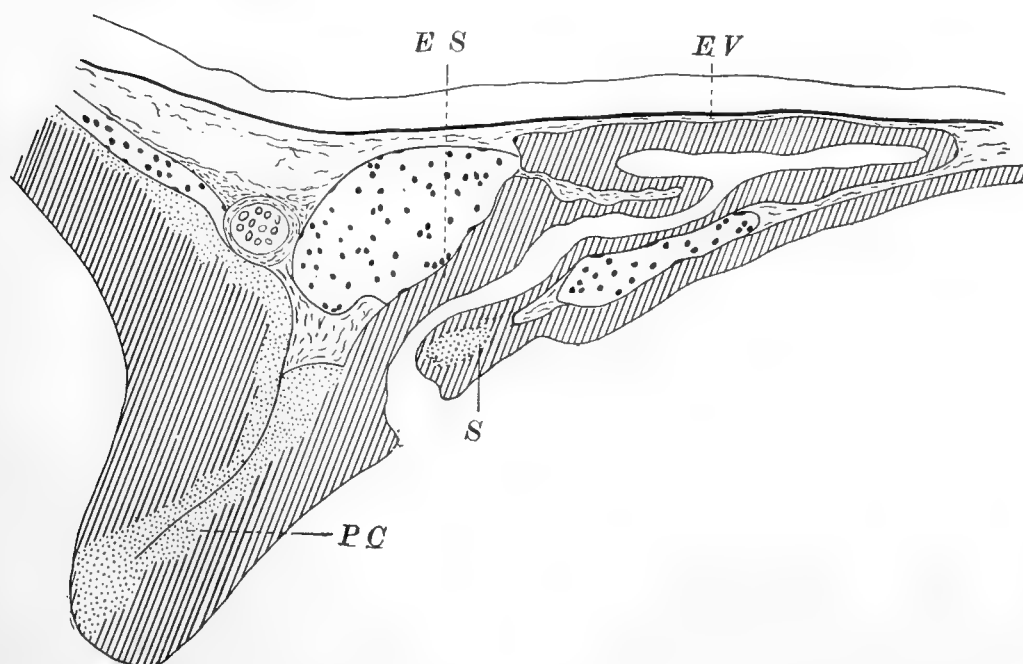


Fig. 20. Median section of the pineal region of *Ameiurus*. Length 10 mm. H.E.C., series no. 388, constructed from sections nos. 89-92.  $\times 290$ .

Dexter ('02), in his study of the development of the paraphysis in the fowl, has identified and figured subdivisions of the fore-brain roof, comparable with those described by Minot; and Warren ('05), who has found the arches in *Necturus*, has shown a very striking resemblance between the embryonic pineal regions of this amphibian and *Acanthias*.

In regard to teleosts it is probable that Minot's subdivisions of the embryonic fore-brain are present throughout the class. In Kupffer's ('06) figure of a trout embryo of 53 days, the epiphysis is represented as an elongate evagination, but the other subdivisions of the the roof are shown to have the form of arches and intervening folds. A post-velar arch and the invaginations of the velum and the posterior commissure are shown in Hill's ('94) figure of *Salmo fontinalis* of 42 days. *Opsanus* embryos present the six subdivisions and also a pars intercalaris. The intercalated part first appears as a distinct segment in embryos of 3.5 mm. lying between the posterior commissure and the epiphyseal arch. As the result of forward growth of the commissure over the intercalated part, the latter as such disappears; that is to say, it no longer remains a segment interposed between the posterior commissure and the epiphysis. It can be recognized, however, in all later stages in the stretch of ependyma underlying the anterior division of the posterior commissure. The fundamental divisions appear less clearly defined than they do in *Acanthias* and, moreover, they are not all evident at the same time as is the case in the dog-fish. In *Opsanus*, the epiphyseal arch is present in the smallest embryo studied as are also the posterior commissure and intercalated part; all three can be seen during a brief period (embryos of 3.5 to 5 mm.). By the time the posterior commissure has grown over the pars intercalaris (embryo of 6.5 mm.), the velar invagination is seen. The epiphyseal arch disappears with the formation of the main epiphysis, the post-velar and paraphyseal arches and the superior commissure presenting themselves at this time (embryos of 8 mm.).

As to the relation which these divisions bear to the neuromeres, no direct evidence was obtained in the present study. Kupffer ('06) has identified the region of Burekhardt's Zirbelpolster (Minot's

post-velar arch) as the median dorsal part of his parencephalic segment which in turn, he derives from the second neuromere. The same author finds the intercalated part to be the roof of his synencephalic segment, derived from the third neuromere. Regarding the relation of the epiphysis to these segments, Kupffer ('06) says: "Es tritt nämlich die Commissura posterior, die man als dorsale Grenzmarke zwischen dem Vorder und Mittelhirne festzuhalten hat, nicht zwischen den Segmenten *p* and *se* auf—hier entsteht die Epiphyse" (p. 175). The segments *p* and *se* are the parencephalic and synencephalic segments. The paraphysis is the product of the telencephalic segment derived from the first neuromere, and the velum marks the dorsal boundary between the telencephalic and parencephalic segments. According to Johnston ('05) the second neuromere gives rise to the optic vesicles; from its narrow dorsal part is formed the velum. The epiphysis, according to this author, belongs to the third neuromere.

### *Epiphysis*

The two epiphyseal outgrowths of *Opsanus* differ in their early form and relations from those of *Salmo*, *Coregonus* and the other teleosts which Hill ('91, '94) studied. In the first place they are not true evaginations but solid outgrowths of the brain-roof. As in the case of the solid epiphysis of *Clupea*, a cavity traversed by fibers is later formed in the main organ. Holt ('91) regarded the fibers as a coagulum and found no eye-like structure in the epiphysis, but Studnička ('05), who looks upon the two walls of the pineal vesicle of *Petromyzon* as retina and pellucida, hints at a comparison of these syncytial nets with the remains of a corpus vitreum of the parietal organ. The network in *Opsanus* is derived, as already shown, from the lightly staining protoplasm occupying the axis of the epiphysis, and it therefore cannot be considered a coagulum of some possible secretion of the walls of the organ. A secretion discharged into the cavity of the epiphysis of *Opsanus* would have no outlet. While the organ is moderately vascular it does not conform in structure with any of the ductless glands. On the other hand there is little evidence

in support of the theory of the epiphysis of this teleost being an ocular organ, either rudimentary or degenerate. Whatever may be the significance of the syncytial network, its formation in *Opsanus* goes hand in hand with the development of the epiphyseal cavity, a process analogous with that which produces the cavities of the central nervous system in the teleosts.

As to the fundamental question of the independence of origin of the two epiphyseal vesicles, the evidence afforded by Hill's material is not convincing. In *Opsanus* the two outgrowths are entirely separate and there is no question of one of them being developed from the other. The smaller bud appears later than the definitive epiphysis and arises from the diencephalic roof. It lies at first to the left and a little in advance of the main organ but secondarily comes into connection with the superior commissure and the post-velar arch. This forward migration recalls the shifting of the anterior vesicle of *Amia* and *Lacerta*.

The end of the epiphysis, from the time it is first seen in *Opsanus*, is closely related with the overlying tissues. In the smallest embryo the cranial mesenchyma extends between the epiphysis and ectoderm. In larger embryos and in larvae, strands of this tissue and finally connective tissue fibers proceed from the epiphysis to the roof of the cranium. There is no evidence to show that the function of these connecting strands is anything more than a passive one in fixing the epiphysis, but the early appearance of a bond between the end-vesicle and the overlying parts is suggestive of some other relation. Dean '95) has expressed the opinion that the epiphysis of fishes is connected with the innervation of the sensory canals of the head, at the same time opposing the theory of its relation to a median eye. In the present study attention was specially directed to a search for evidence in support in this view, but there was nothing observed which pointed to a relation between the epiphysis and the lateral line system, beyond the fact that the former is at one time directed toward the supraorbital commissural canal and is approximated rather closely to it.

The forward inclination of the epiphysis, occurring at the time when the roof of the cranium is first laid out in the mesenchyma,

points to the influence of cranial development on the position of this organ.

The statement made by Goette ('75) that the pineal body in *Bombinator* arises from a bridge which connects the brain and ectoderm has repeatedly been reaffirmed and denied. Van Wijhe ('83) and Hoffmann ('84) have presented evidence in support of the connection; Mihalkovics ('77), Balfour ('85), and later investigators assert that the epidermal bridge has no existence. Locy ('93) claims that the beginning of the epiphysis in the shark can be seen in the medullary plate. No recent investigator has found a continuity between the epiphysis and ectoderm after the formation of the medullary tube, although a close relationship has often been observed between the end of the pineal body and the outer germ layer. This condition and the absence of any intervening mesoderm led Hoffman to believe that the epiphyseal anlage was laid out before mesodermal formation had commenced. In *Opsanus* of 3.5 mm., when the epiphysis is just discernible, a mesenchymal stratum stands between it and the ectoderm and there is no evidence of continuity between these parts.

#### *Posterior Commissure*

It was shown in the descriptive part of this paper that the posterior commissure, arising in the ectoglia layer of the brain-roof, is, at first, located posteriorly to a pars intercalaris, that it spreads forward over this region and finally becomes included within the fold that intervenes between the mid-brain and diencephalon. Moreover, it was found that the commissure in the older embryos, in all stages, and in the adult fish, presents two distinct divisions, anterior and posterior, separated by a connective tissue septum. This mode of development has been observed in other bony fishes, to which reference has been made above (p. 336), and its division into strata has been described and represented in figures of the teleostean brain. It appears, therefore, that a type of posterior commissure appears among bony fishes, characterized by the presence of two strata of fibers separated by a partition. The commissure lies neither altogether in the wall

of the mid-brain nor in the diencephalon, but is so situated that its posterior layer stands in connection with the former, while its anterior stratum is spread out in the roof of the latter caudad to the epiphysis. In form and position, therefore, it differs from the corresponding tract of the elasmobranchs, in which fishes it has been shown to be a fiber bundle associated wholly with the midbrain (Ehlers, '78; Edinger, '99; Minot, '01). With this difference in the commissures of the two classes of fishes is correlated the difference presented by the pars intercalaris, which is extensive in the teleosts, small or absent in the elasmobranchs.

Regarding a pineal nerve, the evidence was insufficient to warrant the statement that such a structure is present in *Opsanus*. There is that close relationship between the posterior commissure and the epiphysis which has been described by Edinger ('99) in *Scyllium* and sturgeon, by Kupffer ('93, '06) in the trout, and shown by Dean ('96) in his figure of *Amia* (Comp. fig. 11). In the smaller embryos of *Opsanus* the posterior commissure extends further upon the base of the epiphysis than it does in the adult, recalling the relation of a tractus pinealis. Many fibers were followed to the base of the epiphyseal stalk but their terminal relations were not discovered.

#### *Superior Commissure*

The observation made by Osborn ('84) on the position of the superior commissure in front of the epiphysis has been many times confirmed and in recent years emphasized by Minot ('01) and Dexter ('02). Cameron ('04), however, appears unwilling to concede that the commissure should be considered as closely related to the epiphysis. He states that in all vertebrates it is situated behind the root of the choroid plexus of the third ventricle. The definition of the position of the commissure given by Osborn, Minot and Dexter implies a topographical relation to a constant organ. The structural relationship between the commissure and the epiphysis in the ganoids described by Herrick ('91) and by Eycleshymer and Davis ('97) is another reason, beyond that of mere topography, for associating the two parts. Yet, the position

of this fiber bundle immediately in front of the epiphysis does not always obtain, as Cameron points out, and a definition of its relations applicable to all cases must wait until more observations have been made. One relation appears to be constant, namely, that the commissure is associated with the post-velar arch. This relationship is shown in median sagittal sections of the brain where it appears that the diencephalic roof in front of the epiphysis is composed of two strata, the cellular ependyma and the narrow fiber layer of the superior commissure. When these are followed in a direction away from the median plane they are found to pass over to the intermediate tubercles. Here all three of the fundamental layers of the brain wall, as recognized by His ('89) and Minot ('92, '03) can be seen, the Randschleier or ectoglia layer being formed by the fibers of the superior commissure. The latter may therefore be regarded as the ectoglia layer of the post-velar arch, small and limited to the posterior aspect of this region in *Opsanus*, but more extensive in *Amia* and *Petromyzon*. The origin and early disposition of the posterior commissure would warrant the same conclusion respecting its relation in the brain wall.

#### *Post-velar Arch*

The post-velar arch reaches its highest development in *Opsanus* when the embryos are about 8 mm. long, and subsequently disappears, probably by incorporation with the velum. The epithelium of the arch is like that of the median lobe of the velum and probably functions as a secreting surface. Hill ('94) and Leydig ('96) have found that this part of the brain-roof in teleosts is specially differentiated to form ridges and secondary folds of the epithelium resting upon a connective tissue foundation, in some cases vascular. Leydig states that in the trout the cells are higher toward the summit of the arch. The great expansion of the post-velar region in the ganoids is well known through the writings of Balfour, Huxley, Wiedersheim, Goronowitsch, Wilder and others in recent years. In *Amia* and *Lepidosteus*, Kingsbury ('97) found the dorsal sac, velum and metaplexus lined with an



ependymal epithelium which appears to consist of secreting cells, and Herrick ('91) describes the pouch of the diatela as possessed of a wall composed of a single row of cells with long cilia or flagella. It appears, therefore, that in these ganoids the epithelium of the arch is especially modified and lines a great evagination of the diencephalic roof. Regarding the elasmobranchs, Minot ('01) says ". . . . the post-velar arch remains small, hence the velum seems to arise later very close to the mouth of the epiphysis."

The connection of the smaller epiphyseal bud with the post-velar arch by forward shifting brings up the question of its possible relationship with those large outgrowths of this region which have been observed by Schauinsland (See Kupffer, '06) in *Callorhynchus*, by Gierse ('04) in *Cyclothone*, and by Handrick (See Studnička, '05) in *Argyropelecus*. Commenting on the latter, Studnička, ('05) says: "Der Fall ist sehr wichtig, da er zeigt, dass der *de norma* breitere Dorsalsack unter Umständen sich in ein enges schlauchförmiges Gebilde verwandeln kann. Dieselbe Erscheinung kann man bekanntlich auch bei der Paraphyse beobachten; auch diese tritt einmal als ein enger Schlauch, ein anderes Mal wieder in der Gestalt eines breiten Sackes (Paraphysealbogen—Sedgwick Minot) auf."

### *Velum Transversum*

The velum transversum of the teleosts, according to the current descriptions, consists of a simple transverse fold of the fore-brain roof, having smooth surfaces and a free ventral margin. Rabl-Rückhard ('82) regards the velum as the starting point, phylogenetically, of the choroid plexus, although he found no differentiation in this direction in the bony fishes. In them this organ seems to be less advanced in its development than it is among the selachians, where it has been shown that projections are formed on either side which are regarded by Minot ('01) as the anlagen of the choroid plexuses of the lateral ventricles. In *Acanthias* a superficial coat appears upon the ependyma of the anterior surface of the velum, the nature of which is uncertain, but as

Minot says ('01, p. 91), “. . . suggests . . . the formation of secretory spherules.” Gentès ('08) regards the velum of torpedo as a true choroid plexus. The enormous development of the velum of Opsanus is a striking peculiarity of the brain of this bony fish. The more important structural characters which have been described on p. 342, seen in all the adult and larval specimens, are those belonging to the true choroid plexus and establish the velum of the toad-fish as such an organ.

Among the teleosts possessing a rudimentary velum, *Belone acus* has been cited by Studnicka ('05). In the brain of the *Ameiurus* embryo, shown in fig. 20, the velum is rudimentary and there is a further resemblance to *Belone* in the peculiar form of the epiphysis. This consists of a large flattened end-vesicle supported upon a rather slender and tortuous stalk. As to the significance of the primitive velum the view recently expressed by Johnston ('09) is interesting, namely, that the velar invagination begins early “on account of the withdrawal of material from the alar plate to form the optic vesicle.” With such a relationship between these structures, some variation in the development of the optic vesicle might be expected in those animals where the velum is rudimentary or absent.

### *Paraphysis*

The occurrence of a paraphysis in a number of teleosts, as discovered by Burckhardt and Studnicka, lends support to the interpretation that has been given to the fold of the fore-brain roof in *Opsanus*. The higher epithelium of the fold differentiates this rudimentary organ from the tela anterior to it, and its position, immediately in front of the velum, corresponds with the location of the paraphysis in all forms in which it has been observed. This relation of paraphysis and velum or choroid plexus is responsible for the identity of the former organ remaining hidden for so long a time. In *Opsanus* the paraphyseal fold is clearly no part of the velum for it appears after the latter has been formed, differs from it in structure and has only a brief existence.

The part of the embryonic fore-brain roof which is to give rise

to the paraphysis has usually the form of a dome. Since Minot ('01) named it the parapyseal arch and demonstrated that the paraphysis arises from it, its presence has been recognized in all the vertebrate classes. (Comp. Dexter, '02; Warren,<sup>4</sup> '05; Kerr, '03; Johnston, '09).

### III CONCLUSIONS

1. A. The six morphological divisions of the fore-brain roof recognized by Minot are present in *Opsanus*, and probably also in *Salmo*, *Salvelinus* and *Amia*.

B. These divisions, in the form of arches and alternating projections into the ventricle, are not all present at the same time in *Opsanus* as in *Acanthias*.

2. A. A pars intercalaris is to be seen as an independent segment of the brain-roof between the posterior commissure and the epiphysis in embryos of *Opsanus* 3.5 mm. long.

B. In the adult it remains as a thick stretch of ependyma, supporting the anterior stratum of the posterior commissure.

3. A. There are two epiphyses connected with the brain of the toad-fish, one of them being a mere rudiment.

B. The main epiphysis lies in the mid-line and develops a stalk and end-vesicle.

C. The rudimentary organ makes its appearance some time after the definitive epiphysis is differentiated, lies at first to the left and a little in advance of it and subsequently migrates forward into the region of the post-velar arch.

D. The origin of these organs is entirely independent, the one from the other.

E. Both epiphyses are originally solid outgrowths, the main organ springing from the epiphyseal arch, the rudiment from the diencephalic roof behind the superior commissure after the disappearance of the epiphyseal arch.

F. The cavity which develops secondarily in the end-vesicle

<sup>4</sup> Warren, John. On the paraphysis and pineal region in *Lacerta* and *Chrysemis marginata*. Assoc. Am. Anat. 25th Session. Boston, December 30, 1909.

and stalk of the main epiphysis includes a weak meshwork of protoplasmic processes continuous with the surrounding walls.

G. Continuity between the epiphysis and ectoderm was not observed; the fibers that extend between the end-vesicle and the overlying parts are mesenchymal in origin.

H. There is no parietal foramen and no differentiation of the epidermis of the epiphyseal region in *Opsanus*.

I. A nerve connection between the epiphysis and lateral line system does not obtain.

J. A pineal nerve was not discovered.

K. There are two epiphyses in *Salvelinus*, the chief organ being median in position, the subordinate outgrowth to the left of the former.

4. A. The posterior commissure of *Opsanus* has its origin in the ectoglia of the brain-roof.

B. In teleosts the commissure is divided into two parts, the one associated with the mid-brain, the other with the intercalated part of the diencephalon.

5. A. The superior commissure lies, in the toad-fish, immediately anterior to the base of the epiphysis.

B. It arises in the ectoglia of the diencephalic roof, retains the relation of an ectoglia layer in the brain-wall of the adult and may be regarded as an incomplete ectoglia stratum of the post-velar arch.

6. A. The post-velar arch attains its maximum extent in the embryos of *Opsanus* and early begins to diminish and finally disappears.

B. Its place is taken by the velum in its backward growth.

C. Its epithelium probably becomes incorporated with the velum.

7. A. In the development of the velum of *Opsanus*, a transverse fold and a median lobe are formed, the latter differentiating as a choroid plexus of the fore-brain ventricle.

B. The velum of *Amieurus* embryos is rudimentary.

8. A rudimentary paraphyseal organ is developed from the paraphyseal arch, appearing later than the epiphysis and disappearing during the early larval life of the toad-fish.

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## REFERENCE LETTERS

<i>A</i>	anterior epiphysis	<i>PA</i>	paraphyseal arch
<i>C</i>	commissural canal of the lateral line system	<i>PC</i>	posterior commissure
<i>D</i>	diencephalon	<i>PI</i>	pars intercalaris
<i>DI</i>	diverticulum of the telencephalic ventricle	<i>PV</i>	post-velar arch
<i>E</i>	epiphysis	<i>S</i>	superior commissure
<i>EA</i>	epiphyseal arch	<i>T</i>	telencephalon
<i>ES</i>	epiphyseal stalk	<i>TR</i>	telencephalic roof
<i>EV</i>	epiphyseal end-vesicle	<i>V</i>	velum transversum
<i>I</i>	intermediate tubercle	<i>VM</i>	median lobe of velum transversum
<i>M</i>	mesencephalon	<i>VL</i>	transverse fold of velum transversum.
<i>P</i>	paraphysis		

THE DEVELOPMENT OF THE NINE-BANDED ARMA-  
DILLO FROM THE PRIMITIVE STREAK STAGE TO  
BIRTH; WITH ESPECIAL REFERENCE TO THE  
QUESTION OF SPECIFIC POLYEMBRYONY<sup>1</sup>

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FIFTEEN TEXT FIGURES AND NINE PLATES

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<sup>1</sup> Contribution from the Zoological Laboratory of the University of Texas.  
No. 105.



## I. INTRODUCTION

*A. Review of the Literature*

It is not our present purpose to attempt any comprehensive review of the literature dealing with the development of the Edentata, nor even of that treating especially of the armadillos. It seems advisable, rather, to limit our survey to those contributions, a knowledge of which is essential to an understanding of the problem of specific polyembryony.

That certain species of armadillos bring forth at a birth young all of one sex has been known for over a century. According to Azara,<sup>2</sup> a writer of the eighteenth century, the natives of Paraguay and of the Argentine Republic knew that this was true for the Mulita (*Tatu hybridum*). Any observant hunter, who had been fortunate enough to capture a litter or two of young animals in a burrow with the mother, might readily have noted such a unique state of affairs, for the sexes are easily distinguishable.

In the latter part of the nineteenth century Herman von Jhering, ('85 and '86), met with similar statements on the part of the natives of Brazil and was sufficiently interested to attempt a scientific confirmation of what had been until then merely an interesting piece of folklore. Two pregnant females came under his observation, the uterus of each of which contained eight male foetuses, all in exactly the same stage of development. Each foetus was described as having its own separate amnion; but all were surrounded by a common chorion.

These conditions were interpreted in a subsequent paper by the same author as indicating the origin of the several embryos from a single fertilized egg, and it was further assumed from the facts in hand that the splitting of the original single germ into separate embryonic primordia occurred at some period after fertilization. Von Jhering apparently saw nothing more fundamental in this situation than the discovery of a new type of animal reproduction to which he gave the name "temnogenesis." Its bearings on the problems of sex determination and of heredity

<sup>2</sup> Referred to by von Jhering.

were not appreciated. To him however belongs the credit of having discovered specific polyembryony in the *Mulita*.

No attempt was made to secure evidence, either internal or external, of the validity of von Jhering's suggestion until Rosner, ('01), took up the subject in connection with his studies of human monochorial twins. On the basis of a histological examination of the ovaries of one pregnant female of the South American nine-banded armadillo he attempted completely to discredit the idea that the several embryos of a litter arise from a single fertilized ovum. Since his observations strike at the very foundations of the question of polyembryony in the armadillos it seems necessary to review his work in some detail.

The genitalia of two pregnant females were sent to him by von Jhering, and an examination showed that the ovaries of only one specimen were sufficiently well preserved to admit of histological examination. Sections of the other pair of ovaries showed that a large percentage of follicles contained more than one egg. There were in all 52 large follicles: 11 with 2 eggs, 7 with 3, 2 with 4, 1 with 5, and 1 with 7. The two largest follicles contained four eggs, exactly the number necessary to produce the four embryos habitually brought forth in a litter of this species. Since the youngest follicles never contained more than one egg the conditions seen in the older ones must have resulted from secondary fusions of adjacent follicular walls, which subsequently disappeared in such a way as to form a common cavity. The author's figures are evidently accurate representations of actual observations and are calculated to convince the reader. Especially striking is the figure of a reconstruction of a series of sections through a large pluriovular follicle in which each of the eggs has its own thick coating of discus proligerus cells.

Rosner believes that the observed condition of four embryos surrounded by a common chorion is to be explained by the following sequence of events: four adjacent follicles fuse in such a way that four eggs are thrown into a single cavity; on the rupture of this compound follicle the four eggs are discharged simultaneously, descend the fallopian tube held together in a mass by means of their discus proligerus cells, become fertilized, undergo

cleavage and come to a common point of attachment in the uterus; subsequently the contiguous walls of the four blastocysts atrophy and a single vesicular chorion is produced.

Were Rosner's observations a record of the normal conditions in the armadillo ovary the question of specific polyembryony would assume an aspect entirely different from that suggested by von Jhering, and we would need to seek no further for an explanation of the observed conditions. The observation that all the embryos in a litter are of the same sex was summarily dealt with by Rosner who considered it as interesting but in no way connected with the presence of a common chorion. Fortunately however there is now every reason to believe that Rosner's material was pathological or otherwise exceptional, for no subsequent investigator has been able to find in the armadillo ovary conditions such as he described.

Cuenot, ('03), while engaged in the study of the problem of the determination of sex, examined the ovaries of one pregnant and of one virgin female of the species investigated by Rosner. In the ovaries of the pregnant specimen there occurred only one follicle of the pluriovular type and this contained only two small, rather abnormal ova. Out of 119 follicles in the ovaries of the virgin female however three contained two or three eggs, but none was found with the number requisite to give rise to the number of young habitually born in a litter.

Until quite recently no further progress was made toward the solution of the problem. In 1909, however, there appeared almost simultaneously and quite independently, two contributions to the subject, one by Fernandez, ('09), on the *Mulita* (*Tatu hybridum*), and the other a preliminary report by the present writers, ('09), on the North American armadillo (*T. novemcinctum*). The two species evidently agree very closely in many of the more fundamental details of development but differ sufficiently to make it both interesting and valuable, from the comparative standpoint, to have the developmental history of both species worked out in the fullest detail.

Fernandez presents somewhat detailed descriptions of seven rather early embryonic stages and enters upon a brief discussion

of some of the more important questions involved. He was especially fortunate in securing in a good state of preservation two very young embryonic vesicles in which the demarkation of the several embryonic primordia had not yet manifested itself. For the equivalent of this stage we have looked in vain and hence, for the present at any rate, are compelled to rely on Fernandez's description for an explanation of our own earliest stages. Since it is necessary constantly to refer to Fernandez's work in the body of the text no further comment of an introductory character is needed here.

At this point it becomes necessary to refer to our own preliminary report in order to correct the description of fig. 3 in that paper. The specimen there figured was presented to us with the statement that it was intact in every respect, except that the uterus and the contained vesicle had been slit open along the mid-ventral line. On the basis of this statement, together with a study of the external features, we reconstructed the vesicle *in situ*. Our subsequent investigations of fresh specimens has led us to suspect that what we took to be a young vesicle was in reality only the villous portion of a somewhat later stage.

### *B. Material and Methods*

During the past two years we have had the opportunity of examining 137 females of the native armadillo, together with a considerable number of males. During the breeding season hunters employed to collect material for us covered a wide range of territory in south-central Texas. These men were frequently obliged to haul the living animals through rough country for distances of fifty miles or more in order to reach an express office whence they could be shipped to our laboratories. As a rule a number of days elapsed between the capture of the animals and their arrival in Austin. This delay would serve in part to explain our ill success in securing the earliest embryonic stages. In order to obtain a complete series we believe it will be necessary either to breed the animals in captivity or to accompany the hunters on their expeditions so as to lose no time in examining freshly

fertilized females. Although we fully expect to secure the earliest stages in the course of time it seems inadvisable for us to postpone the publication of the results thus far obtained, results sufficiently clean cut in themselves to form the basis of a self-consistent and fairly well rounded embryological account.

At present we have in our possession seventy embryonic vesicles comprising a close series of stages ranging from the primitive streak stage to birth.

Little need be said about the methods employed. To each animal that reached the laboratory was given a number and a page in a ledger where all facts that might be of interest were recorded. In case the carcass was to be thrown away complete records of all data that might be useful in the future were kept. The ovaries of the majority of the females were fixed in the standard cytological fluids. Every part taken from a given specimen was numbered accordingly. Much of the data thus gathered proved useful during the course of the work and we have no doubt that all of it will ultimately serve to throw light on future investigations.

### *C. Purpose and Scope of the Present Paper*

In this our second contribution to the developmental history of the armadillos the main purpose in view is to establish the fact of specific polyembryony and thus to clear the way for future investigation. A more or less tentative explanation of its causes and of the conditions and relations that result from it is hazarded on the strength of the evidence now in hand, which is internal in contradistinction to that derived from an examination of the ovaries and testes, no detailed discussion of which is attempted at present.

Although the question of polyembryony is the central problem it is impossible to treat of it as an isolated phenomenon for the reason that many curious developmental processes are intimately associated with it. The history of the amnion and of the placenta, for example, would be indecipherable apart from the fact of polyembryony, and the inter-relationships of the embryos admit of a rational explanation on no other basis. The associated phenome-

non of germ layer inversion is also (indissolubly) bound up with polyembryony and in turn involves many peculiar and interesting relations.

Any adequate treatment of the principal problem will therefore necessitate the presentation of a somewhat complex array of facts whose combined verdict will, we trust, establish our main contention.

Except in the case of the two earliest stages described no attempt is made to present a detailed account of the organogeny of the species. No doubt such a study would reveal many facts of interest to the specialist in mammalian embryology, but would serve only to cloud the main issue with obscuring details.

## II. THE FEMALE GENITALIA

The uterus is simple and not unlike that of the primates in form. In the non-pregnant condition it varies somewhat in size and shape according to the previous history of the individual. In old females that have produced a number of litters the organ though non-pregnant may be distended to several times its normal size, often leading the observer into the vain hope of finding the earliest stages. The uterus of the virgin adult presents a less modified condition and will furnish a basis for the accompanying detailed description.

The average dimensions of the non-pregnant uterus are as follows: 13 mm. from the tip of the fundus to the junction of the cervix with the vagina, 15 mm. between the points of entrance of the two fallopian tubes, and 10 mm. deep dorso-ventrally. Viewed from the dorsal aspect the uterus appears to be broadly kite-shaped (fig. 7) with the posterior angle blending into the vagina. The fallopian tubes are approximately straight where they enter the uterus, but near the ovaries are strongly convoluted, each ending in a hood-shaped fimbriated infundibulum, which, with the aid of a posteriorly directed flap of the broad ligament, covers a large part of the ovary and thus renders the escape of the ovum into the body-cavity well-nigh impossible. The points

of entrance of the fallopian tubes are about equadistant from the tip of the fundus and the vagina, thus rendering the cavity of the uterine body much larger as compared with that of the cervix than is the case in the human uterus, where the tubes enter practically at the distal end of the organ.

The ovaries are kidney-shaped having the convex side directed anteriorly, with reference to the axis of the animal. In virgin females the two ovaries are approximately equal in size, but in individuals that are or have been recently pregnant there is always a considerable difference in the size of the two ovaries. The larger one may be two or three times as large as the smaller, and this greater size is invariably due to the presence of a single enormous corpus luteum, the actual bulk of which may be much greater than that of the remaining ovarian tissue. There are found not infrequently smaller bodies (resembling in histological appearance the large corpus luteum) which are crowded to one end of the ovary and suggest by their shrunken and irregular form that they are either relics of a previous pregnancy or simply the lutea of ova which were never fertilized. It may be stated without hesitation however that *there is never more than one large and prominent corpus luteum in the ovaries of a pregnant female.*

The mucosa of the uterus is undoubtedly deciduate in character, as may be seen in the illustration of a section taken from a series cut through a pregnant uterus and its contents (fig. 1). Even at the comparatively early period represented it can readily be seen that the mucosa is separated from the outer layers of the uterus by a lymph space of considerable magnitude.

Since the young embryonic vesicle always gains attachment to the mucosa near the tip of the fundus it is not a difficult matter to orient it with reference to the uterine axis. It will be found convenient to refer to the fundus and cervix ends of the vesicle, the former being the original attached and the latter the original free end. The axis of each embryo is also related to that of the uterus, in that its anterior extremity is directed towards the cervix end of the vesicle, except in advanced conditions when the length of the umbilical cord occasionally permits an embryo to reverse its position within its amniotic sac.

The pregnant uterus assumes a variety of shapes in different individuals. At approximately the same period of pregnancy it may be either elongated or comparatively broad, either blunt or pointed at one or both ends, and either simple or clearly bilobed dorso-ventrally at the fundus end (figs. 42 and 43). These various forms are not due to the position or arrangement of the foetuses, which in this respect are practically constant, but probably to individual variation influenced by the previous functional history of the organ.

### III. NUMBER, ARRANGEMENT AND SEX OF THE EMBRYOS

#### *A. Number of Embryos*

In sixty-five out of seventy cases there were four normal embryos in a vesicle. It may be assumed then that four is typical for the species. Three atypical conditions occurred which may be listed as follows:

1. Vesicles containing five normal embryos (three cases, nos. 28, 91, 108).

2. Vesicle containing three normal embryos each measuring 15 mm. and one decidedly abnormal embryo 7 mm. in length (no. 57). No doubt this vesicle was destined to produce a three-embryo litter.

3. A case of twins (no. 137). These were born in captivity. A very careful examination of the uterus and intestines of the mother convinced us that there were no other young born. This may have been a case somewhat like the preceding except that two embryos degenerated instead of one.

There appear not infrequently in otherwise normal embryonic vesicles small amniotic sacs that usually contain the more or less completely degenerated remains of what may once have been extra embryos. In one case (no. 108), a vesicle with five normal embryos, such a sac appeared, which, if truly the representative of an extra embryo, would furnish an example of a six-



embryo vesicle. In another case (no. 17), which is peculiar in several other respects, there occurred a small empty amniotic sac fused firmly to the wall of the Träger and connected with the amniotic sac of a normal embryo by means of an amniotic canal similar to those of the other embryos. In still another case (no. 9) a fairly large sac in the Träger region was connected by means of a perfect amniotic canal with that of a normal embryo (fig. 44). There is little doubt but that these sacs represent the remnants of supernumerary embryos and as such are the equivalent of those described by von Jhering and Fernandez.

It is interesting to note in this connection that *Tatu novemcinctum* shows a stronger tendency toward stability in the number of foetuses in a litter than does *T. hybridum*. There is evident, however, in the latter species, a tendency to produce eight young in a litter, just twice the number typical for our species. The numbers of individuals in a litter ranges, however, from seven to twelve.

### *B. Arrangement of Embryos*

In order to clear the way for the description of the early embryonic conditions it should provisionally be pointed out that the four embryos of this species are arranged in pairs, one pair to each lateral half of the uterus. The upper embryo of the left hand pair usually occupies the dorsal amniotic quadrant and is therefore referred to as the "dorsal embryo" (no. III). The lower embryo of the left hand side occupies the left lateral amniotic quadrant and is referred to as the "left lateral" embryo (no. IV). The lower embryo of the right hand pair occupies the ventral amniotic quadrant and is the "ventral embryo" (no. I), while its mate, occupying the right lateral quadrant is spoken of as the "right lateral" embryo (no. II). Nos. I and II constitute the right hand pair and nos. III and IV the left.

The orientation of the vesicle in the uterus and the arrangement of the four embryos with reference to the vesicle and to one another is rather precise, so that a plane running from the mid-dorsal to the mid-ventral line of the uterus would divide

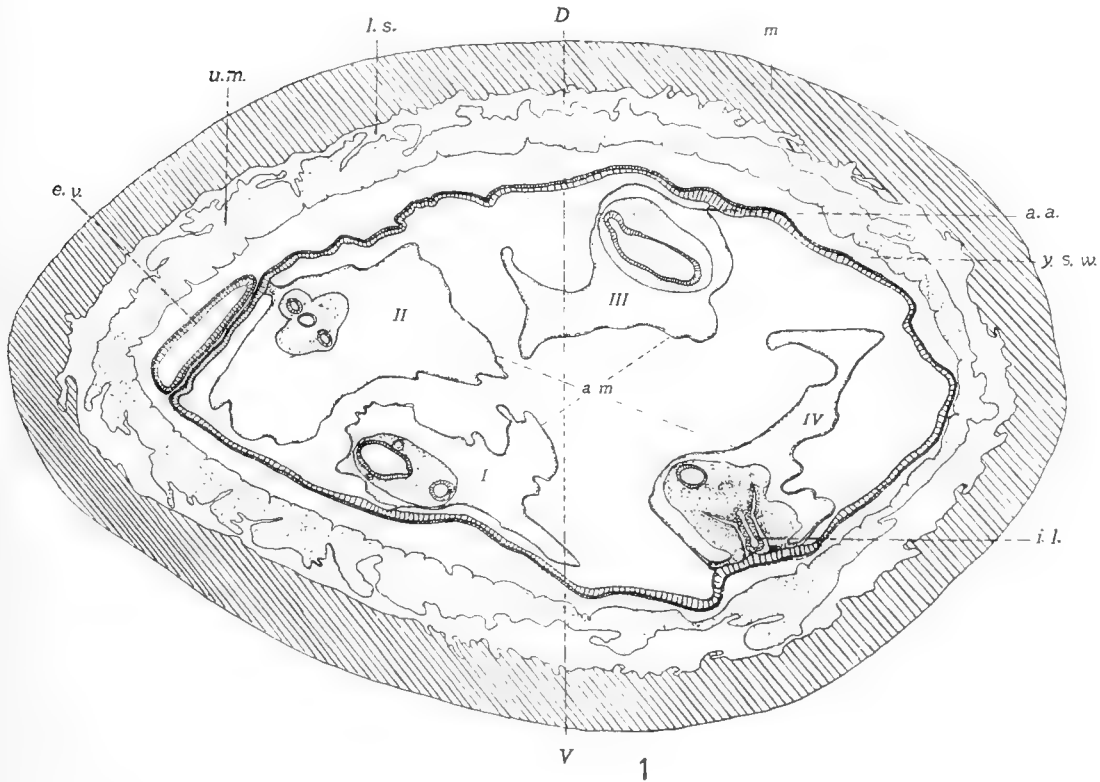


FIG. 1. Outline camera drawing of a transverse section through a pregnant uterus measuring about 15 mm. long by 14 mm. wide. Line D-V is drawn from the points lying at the middle of the dorsal and ventral sides of the vesicle. It divides the section of the vesicle into halves. Embryos I and II lie in the left hand half, and III and IV in the right hand half. *a. a.*, line of attachment of the amnion to the vesicle; *e. v.*, a small extra chorionic vesicle, which is not fused with the larger one; *i. l.*, intestinal loop; *l. s.*, lymph sinus between the wall of the vesicle and the uterine mucosa, *um.*  $\times 9$ .

the two pairs of embryos and their placental areas from each other. There may be a secondary shifting of the positions of the various amniotic sacs, so that in the definitive condition one may find the upper embryo of the right hand pair occupying the dorsal position, which in the great majority of cases is occupied by the upper left hand embryo. Such a shifting might easily occur at any time before the walls of the various amnia fuse firmly with the chorion, a process that does not occur until a late period of gestation. Previous to this time each amnion is attached to the chorion only along a meridional line, an attachment that would permit the whole sac to swing almost as readily to one side as to

the other. Reference to fig. 1 will show that the amnion of embryo II, especially after the amnia have increased considerably in size, might readily overlap the line D-V, so its embryo would occupy the dorsal amniotic quadrant. The same shifting might equally well occur on the ventral side. Such shiftings might take place however without affecting in any way the point of the embryonic attachment, which is immediately adjacent to the original amniotic attachment (fig. 1, *a.a.*). Such departures from the typical arrangement of embryos in the vesicle are rather rare, and are not to be considered as of prime importance, for they in no way affect the pairing of embryos, a relationship depending on the point of attachment of the latter which is equivalent to their point of origin. The significance of this arrangement is discussed in a subsequent chapter.

### *C. Sex of Embryos*

In thirty-eight embryonic vesicles the foetuses are sufficiently advanced to permit of the accurate determination of their sex. There is no exception to the rule that *all embryos in a vesicle are of the same sex.*

Although the armadillo hunters claim that males are considerably more numerous than females we find no inequality of sexes in the sets of embryos in our collection, exactly half of which are male and half female. In the small collection of nine advanced sets of mulita embryos Fernandez found that six were female and three male. On this basis he proceeds to discuss the significance of the apparent disproportion of sexes in the species. No doubt a larger collection of embryonic sets would have shown no such disproportion, for in our earlier survey of the subject of sex distribution we found a much larger proportion of males.

## IV. THE EARLY EMBRYOLOGY

In the development of the nine-banded armadillo we find that striking peculiarity, met with in the rodents, of germ-layer inversion. In the case of the armadillo the inversion is intimately bound up with the formation of the four embryos, and without it the mechanics of specific polyembryony, as found here, would be inexplicable. The possession of a common amnion by the embryos at an early stage could only occur as a sequence to inversion, and strongly suggests that the embryos are the product of a single fertilized egg.

In the present description of *Tatu novemcinctum* we shall begin with the primitive streak stage, and leave out of account the younger embryos (except for a brief reference to the work of Fernandez) until we shall have secured a series covering that important period. In dealing with the following stages considerable emphasis is placed upon the embryological details, and especially upon the relations existing between the embryos. This is done because these stages furnish the strongest internal evidence for polyembryony that has been brought forward.

A. *The Earliest Stages of Fernandez*

It will be necessary to refer to the work of Fernandez, especially to the part in which he describes his youngest two stages; because they hold the key not only to the morphology of the older embryos of *Tatu hybridum*, but also, we believe, to that of the stage of *T. novemcinctum* which we are about to consider.

Fernandez secured two specimens of his earliest stage, and the one he describes in detail was cut longitudinally into twenty-three sections (10 microns thick). It was found attached to the mucus membrane at the bottom of a fold at the fundus end of the uterus.

Fernandez correctly interprets the condition presented in this early stage as one having been brought about through the process of germ-layer inversion, and compares the vesicle to corre-

sponding stages of the rat and the mouse, described respectively by Selenka, '84, fig. 29, Taf. XIV., and Melissinos, '07, figs. 38 and 39 Taf. XXXIV. He thus finds the vesicle composed of three sacs lying one within the other: the innermost one is the ectoderm, the middle the entoderm, and the outer the trophoblast (hinfälligen Ectoderm), which at the proximal or attached end of the vesicle is differentiating into the Träger. The similarity between the vesicle of Fernandez and those figured by Melissinos (his figs. 38 and 39) is particularly striking, though, as he points out, there are several differences. In the first place, the mesoderm is not yet formed and the so-called Träger cavity scarcely can be regarded as homologous with that of the mouse. In the second place, the parietal layer of the yolk-sac entoderm is not complete, but is wanting in the distal portion of the trophoblast. If, however, we may be allowed to make a suggestion based on a study of his photograph (fig. 6, Taf. XIX), what appear to be scattering cells lying along the inner surface of the distal trophoblast might well be interpreted as representing the remains of the parietal layer of the yolk-sac. This would make this early stage of the *Mulita* very closely resemble the corresponding stages of several other forms, as illustrated in the figures of such investigators as Selenka ('84), Robinson ('92), Jenkinson ('00), and Melissinos ('07).

The most interesting portion of this young vesicle of the *Mulita* is the inner sac, for it is the primordium out of which the ectoderm of the several embryos later differentiates. Fernandez points out the significant fact that it gives no indication of being a multiple structure, such as one would expect to see if the vesicle were the product of the fusion of several eggs.

The second stage of Fernandez is decidedly more advanced than the preceding, and was found lying loose in the fundus end of the uterus. In the preserved condition it measured 3 mm. long by 2.3–2.5 mm. wide. The general condition of the germ layers in this vesicle is made clear in the slightly modified copy of his second text-figure (fig. 2). The figure, which is a diagram of a median longitudinal section passing through two embryos, is shaped like a horse shoe. The entire convex anterior and lateral

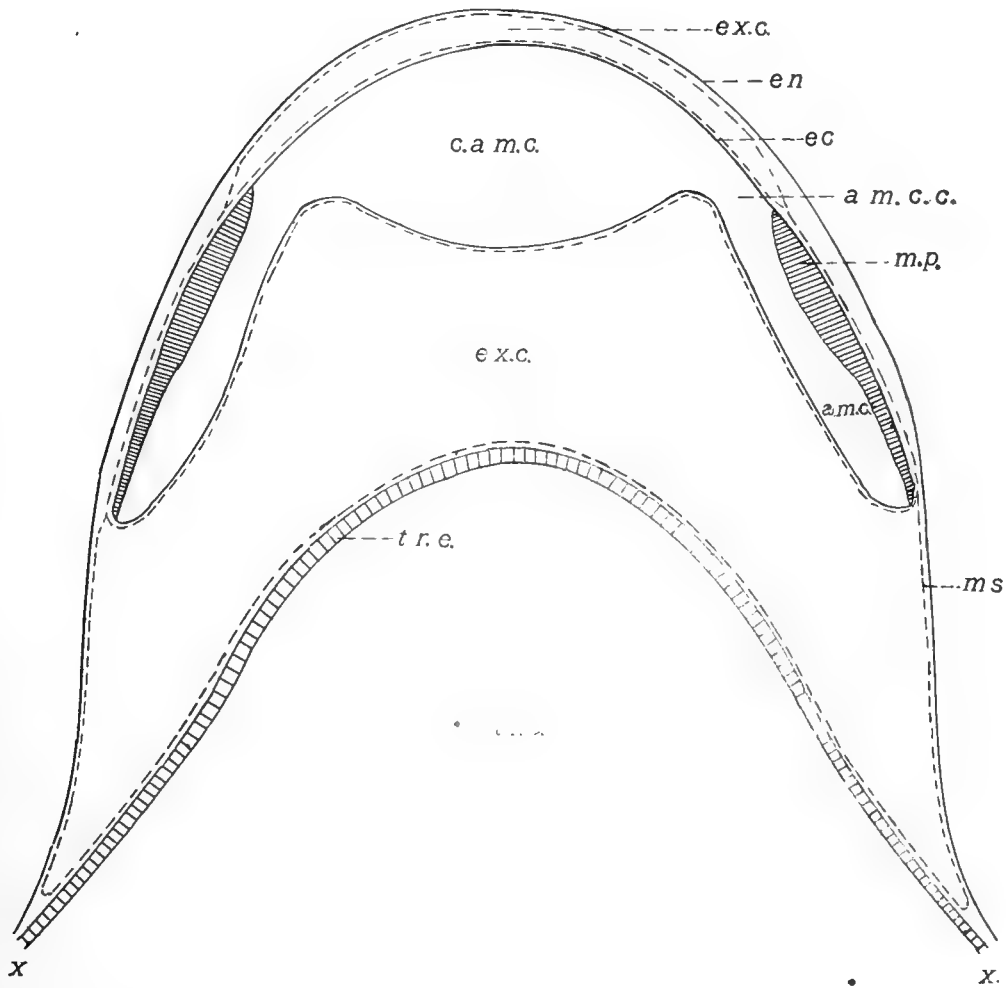


FIG. 2. A diagrammatic longitudinal section of an early stage of the *Mulita*. *ex.c.*, extraembryonic body cavity; *en.*, entoderm; *am.c.*, amniotic cavity of the embryo; *am.c.c.*, beginning of the amniotic connecting canal; *c.a.m.c.*, cavity of the common amnion; *ms.*, mesoderm; *m.p.*, medullary plate; *tr.c.*, Trager cavity; *tr.e.*, Trager epithelium; (slightly modified after Fernandez).

margins represent the entoderm of the inverted yolk-sac, while the concave posterior margin is covered with Trager epithelium. Between these two regions occurs a narrow zone where the vesicle was attached to the uterine wall (marked X).

The Trager cavity (*tr. c.*) is situated in the concave space roofed over by the Trager epithelium. While in some respects this cavity is comparable to that of the rodents, yet for the most part any such comparison would appear to be strained. The difficulty standing in the way of pointing out any true homologies, however,

must be attributed to the incompleteness of the history of these early stages—a fact which Fernandez freely admits.

Within the limits of the vesicle there are two distinct cavities: one the general cavity of the vesicle (*ex. c.*), and the other the common amniotic cavity (*c. am. c.*). The former is lined throughout with mesoderm, and the latter with ectoderm.

The embryos, which are in the medullary plate stage, lie in pocket-like diverticula from the lateral margins of the floor of the common amnion; and each embryo is connected with the latter by a short tube, which is the beginning of the amniotic connecting canal. The common amnion, together with its accompanying embryos, is the product of the inner ectodermal sac of the earlier stage. It is not at all easy to explain fully the manner in which the various structures presented in this vesicle develop out of the primordia of the preceding vesicle, although the history of several of them is self evident. To go from this to the succeeding stage is, however, an easy step, and we shall therefore pass directly to it as exemplified in our youngest vesicle of *Tatunovemcinctum*.

### *B. The Primitive Streak Stage*

We were fortunate in being able to secure from the uterus the entire embryonic vesicle in practically a perfect state of preservation. The opportunity was thus afforded not only to make a detailed study of the relations existing between the different embryos but also to obtain a drawing of the vesicle as a semitransparent object (fig. 12). In the preserved condition it measured 7 mm. wide by 9 mm. long. It is slightly flattened dorso-ventrally but in general outline is shaped like an inverted balloon, with two lateral horn-like projections which fit into the openings of the fallopian tubes. These horns persist for a considerable time and are of great service in aiding one to maintain the correct orientation of the vesicle during its early development.

The surface of the vesicle presents two distinct regions, the lower of which fits into the fundus end of the uterus and is recognized as the *Träger*. It is therefore covered by *Träger* epithe-

lium. At the extreme lower end there is a small cap-like area where the primitive attachment knots or cords of the Träger epithelium are beginning to disappear. The other region occupies the upper two-thirds of the vesicle and differs from the preceding both in its greater transparency and in the complete absence of a trophoblast. This region is the yolk-sac of the inverted type, and consequently is covered with the entoderm. It is rather indistinctly divided into two portions: (1) the central zone occupied by the embryos and their vascular areas, and (2) the cap-like upper third in which the almost complete transparency is obstructed by the presence of the common amnion and its connecting canals.

Two of the embryos lie on the upper side (corresponding to the ventral side of the uterus) and two on the lower side of the vesicle. Each embryo is connected with the Träger region by a rather broad band, the belly-stalk, and is surrounded by an amnion. Since there is an inversion of germ layers, the embryos when viewed from the outside of the vesicle are seen from their ventral aspects; hence, the posterior portion of each amnion is invisible except as seen through the semi-transparent embryo. Anteriorly, however, the lateral margins of the amnia are clearly distinguishable and are seen to pass forward as the tube-like, amniotic, connecting canals. These lie on the inner or mesodermal surface of the yolk-sac, to which they are loosely attached, and in passing forward they converge and finally enter the common amnion. They do not communicate with this by four distinct openings, but by two, for just before reaching it, the canals belonging to the dorsal and left lateral embryos unite to form a single tube, as do also those belonging to the ventral and right lateral embryos. As will be pointed out in another section, this fusion of the canals is an indication of the pairing of the embryos since the union in each case is between individuals of a pair.

The common amnion at this stage is a comparatively small vesicle lying at the extreme cervix end of the vesicle. The manner in which this condition has been evolved from that seen in the second stage of Fernandez is not difficult to figure out. On the one hand, the cavity of the embryonic vesicle has undergone



an enormous extension, due in part to the natural growth of the vesicle and in part to the modification in the shape of the Träger wall, which has changed from concave to convex; on the other hand, the common amnion not only has failed to keep pace with this rapid expansion of the embryonic vesicle, but has actually ceased to grow at all, and is destined soon to degenerate and disappear. In the rapid growth of the embryonic vesicle the embryos gradually have been drawn away from the common amnion, and consequently their connections with it have been pulled out into the long, slender, tube-like canals.

The embryo viewed from the dorsal side shows the exact relations existing between it and the amnion (fig. 13). In general outline the embryo is slipper-shaped and throughout the greater part of its length the amnion conforms to this contour. Both anteriorly and posteriorly the amnion narrows down rapidly—in the former direction to produce the amniotic canal (*am. c. c.*) and in the latter to form the posterior amniotic process (*p. am. .p.*), which ends blindly above the Träger. The level at which the amnion becomes narrower than the belly-stalk varies in different embryos. In the embryo in question it cuts in some distance posterior to the mouth of the allantois, but in other cases it may cut in at a level somewhat anterior to this point.

The entire embryo, from the anterior end of the medullary plate to the posterior tip of the amnion, measures 3.5 mm., but the embryo proper is only 2.5 mm. long. Running through the central part of the medullary plate is the elongated primitive streak, in which is a well developed primitive groove with a faintly defined primitive pit at its anterior end. The primitive streak is exactly 1 mm. long, and has at its anterior end a distinct head process measuring 0.28 mm.

The outline of the allantois is seen through the embryo, and begins a short distance back of the posterior end of the primitive streak and extends through the mesoderm of the belly-stalk, finally ending some distance anterior to the tip of the amnion. Fernandez does not describe the development of the allantois in the *Mulita*, and this stage is, of course, too far advanced to give any clue to the exact nature of its origin.

Lateral to the embryo is seen the beginning of the yolk-sac or vitelline circulation. At this time the blood islands are well developed and incipient blood vessels are represented by a network of anastomosing cords of mesoderm. About midway between any two contiguous embryos there is a band-like area extending from the Träger to the upper limit of the area vasculosa. The band represents the region where the boundaries of the vascular areas of adjacent embryos come together, and thus corresponds to the sinus terminalis of other forms, except that it is double in composition. At the anterior margin of the vascular area of each embryo the sinus terminalis tends to form the arc of a circle, a tendency which, if not inhibited by the crowding of four embryos, would result in the production of a circular sinus exactly as in other forms. As a result of this retardation by crowding the anterior margin of the vascular zone of the four embryos is in the form of a series of scallops.

For an appreciation of the condition of the germ layers it is necessary to turn to a study of representative sections. In the most typical of these, such as that taken through the primitive pit, the neural portion of the ectoderm is thick and has the general appearance of that of corresponding stages of other forms (fig. 19). The outer ends of the section curve decidedly upward, especially the one on the right, but for the most part this is due to the fact that the embryo conforms to the general curvature of vesicle. At the ends of the section the medullary plate turns upward to form the amniotic ectoderm, which is composed of a single layer of cells.

In the central part of the section the entoderm is composed of rather flattened cells, which, however, remain distinct from the overlying mesoderm. Beyond the limits of the primitive streak it becomes thicker and its cells are cuboidal in shape. It must be kept in mind that the entoderm actually forms the outer surface of this region of the vesicle; for the trophoblast has practically disappeared and there are found only a few of its cells scattered here and there along the outer surface of the entodermal layer.

The mesoderm is arising from the primitive streak region in the characteristic manner, and laterally it thins out and, at the point where the ectoderm turns up to give rise to the amnion, divides into two layers, one following closely the amniotic ectoderm and the other the yolk-sac entoderm.

Through the middle of the head process (fig. 18 *h. p.*) the entoderm at the center of the section is barely distinguishable from the mesoderm, and in many places the union of these two layers is very intimate. This must be looked upon however as a condition which is in all probability secondary. In the region of the head process proper the mesoderm cells are closely packed together, but are entirely separate from the neural plate.

Anterior to the head process the mesoderm rapidly thins out practically to a single layer of cells and is easily distinguishable from the entoderm (fig. 17).

Anterior to this section the mesoderm passes into a thickened region of the entoderm, which obviously has nothing to do with the mesoderm, but owes its existence to a proliferation of entoderm cells (fig. 16, *p. p. h.*). It was not detected in the whole mount preparations of the embryos, but its extent is easily determined by a study of sections. The thickening runs through the first five sections beginning with the anterior tip of the embryonic shield, and its width is equal to its length, and it therefore forms a circular plate about 45 microns in diameter. In every respect this circular spot corresponds to the "protochordal plate" of Hubrecht, ('08), who has laid especial emphasis upon it as a region where the entoderm is clearly a source of mesoderm formation. Whatever may be one's conviction regarding Professor Hubrecht's interpretation one can at least be certain that the thickening is purely of entodermal origin in this species. Our series is here too incomplete to permit of tracing out the history of the protochordal plate, and thus to see whether its definitive condition is simply that of mesoderm formation, or whether it contributes to the formation of the fore-gut or the oral plate.

It should be stated here that the protochordal plate at the stage under discussion thins out to a single layer towards its margin,

where it gradually passes into the surrounding entoderm. In many places the mesoderm cells are beginning to migrate in between the plate and the ectoderm, and especially is this true in the more anterior sections (fig. 21). In this section, which shows six of the mesodermal cells, the anterior limit of the protochordal plate is represented. A very short distance in front of this the sections pass through the amniotic canal (fig. 20), which is seen to be composed of two layers, a rather thick inner ectodermal layer, and a thin outer mesodermal layer. In some places the canal is loosely connected with the underlying mesoderm of the yolk-sac, but for the most part it merely lies in contact with the latter.

In sections lying posterior to the primitive pit there is nothing of especial note until we come to the region where the allantoic tube takes its origin. The mouth of the allantois is in the form of a deep groove traversing the ventral side of the anterior end of the belly-stalk (fig. 22, *al*). This is lined with an especially thick entoderm and gradually fades out anteriorly, but posteriorly suddenly narrows down to form the tube. The mesoderm of the belly-stalk appears to extend laterally to form the two wing-like processes, which are to be interpreted as representing cross section of the belly-stalk bands (*b. b.*). Externally these are covered with an epithelium, but within are composed of a loose mesodermal tissue in which run the umbilical blood vessels together with their accompanying sinuses. In section the posterior amniotic process is triangular in shape, and is not much more than half the width of the belly-stalk.

In sections taken through the posterior end of the embryo (fig. 23) the allantois is reduced to a slender tube, having a small lumen. The amnion is here triangular in cross section with the lower angle coming in close proximity to the allantoic entoderm. The mesoderm has much the same shape as in the preceding figure, but may be divided rather indistinctly into two portions: (1) the allantoic mesoderm which surrounds the entodermal tube, and has the cells compactly arranged; (2) the more distal wings or belly-stalk, bands through which the blood vessels run.

The semidiagrammatic longitudinal section of the primitive streak stage is shown in fig. 24, and in connection with what has

been said above concerning the transverse sections, this may be studied with profit. The entoderm in this section can be traced from the protochordal plate back along the entire length of the embryo. Throughout the greater part of its length it is composed of flattened cells, but near the posterior end of the primitive streak these cells become cuboidal, and in the region of the mouth of the allantoic tube (*al*) take on a columnar appearance. Posterior to the allantoic opening the yolk-sac passes back and ends abruptly at the margin of the Träger epithelium (*tr. e.*).

While the median section does not show the lateral belly-stalk bands which form the main connections between the embryo and the Träger, it does, however, bring out with clearness the union between these two as seen at the extreme tip of the embryo. This connection (*ms. co.*) is simply a backward and downward continuation of the allantoic mesoderm, which passes over into the general mesodermal lining of the Träger region.

### C. *The Five to Seven Somite Stage*

The general relations existing between the various parts of the embryonic vesicle in this stage closely resemble those of the primitive streak stage, but the vesicle is almost twice as large, measuring 15 mm. long by 14 mm. wide (fig. 14). Owing to this increase the horns are not only relatively but actually shorter than in the preceding stage. The Träger has undergone marked differentiation and shows a tendency to overgrow the yolk-sac region. The common amnion with its canals presents the same general features as before.

The most interesting changes have occurred in connection with the development of the embryos, and it is to these that we would direct attention. In the first place emphasis should be placed upon the fact that the embryos are not equally differentiated, for the dorsal and left lateral have each, five pairs of primitive segments while the ventral and right lateral embryos have seven. In other words, the individuals of the same pair are in the same stage of development.

In the five somite embryo (fig. 30) the neural folds have not yet coalesced to form the brain vesicle, and consequently the neural groove is open throughout its entire length. The posterior ends of the neural folds embrace the much reduced primitive streak. The embryos are bounded laterally by an area pellucida, which is rapidly being invaded by the blood cords.

In sharp contrast to this embryo is the individual from the other pair showing seven somites (fig. 31), and unless one were from the first aware that they were members of the same set of embryos, one would not so classify them. There are really only six and one-half somites in this embryo, for the most anterior or cephalic pair is connected with the head mesoderm and is somewhat smaller than the succeeding pairs (fig. 15). There is a slight indication of an eighth pair being cut off from the anterior end of the unsegmented paraxial mesoblast.

The amnion has undergone several marked changes, chief among which are (1) its enlargement in the cephalic region of the embryo and (2) its reduction in width at the level of the distal part of the belly-stalk. In this stage the neural folds have risen up and coalesced to form a portion of the neural tube. The point where the fusion first occurs is at the level of the mid-brain region, and from this place it progresses both backwards and forwards. The anterior progress of the union, however, takes place rather slowly and the final closing on the under side of the fore-brain to form the neuropore does not occur until a period much later than this.

At the posterior end of the diverging folds the reduced primitive streak is seen as a broad plate, which in the mid-ventral region is slightly concave, and by transmitted light appears to be decidedly thicker than the lateral portions. The notochord is seen to arise from the anterior end of the primitive streak and to extend forward between the folds. At the point of origin of the notochord the primitive streak is unusually thick, forming a distinct primitive knot, just back of which is the suggestion of a primitive pit. At the posterior end of the primitive streak the entodermal allantois is faintly visible. It extends backward lying beneath the floor of the posterior amniotic process, and falls far short of reaching the tip of the latter.

The belly-stalk now shows a tendency to form into two bands at the proximal or attached end. Each band later carries an umbilical artery and vein from the placental disc to the embryo, that is, they form the attachment of the umbilical cord to the wall of the vesicle. The anterior margins of the bands are turned up to form scroll-like structures beyond which the scale-like villi of the Träger are beginning to extend out over the yolk-sac (fig. 15 s. v.).

There is yet to be considered the yolk sac circulation. This consists of a net work of anastomosing mesodermal cords, which in section are seen to be composed of a central mass of incipient blood cells, surrounded on the upper side by an attenuated layer of mesoderm and on the lower by the entoderm (fig. 8, b. c.). These cords do not become hollowed out even at a much later period than this. Indeed it is doubtful whether they ever become functional blood vessels.

In considering the details of structure we shall confine our accounts to a brief description of a series of transverse sections of the five somite embryo, and to the median longitudinal section of a seven somite embryo.

In the region of the neural fold the neural groove has become greatly deepened to form the first rudiment of the brain vesicle (fig. 26, n. g.), and the lateral margins of the medullary plate have become tucked in beneath, thus forming a bay on each side that is at once recognized as the lateral extensions of the head-fold (h. f.). In consequence of this folding the extreme lateral portions of the amniotic cavity have had the marginal parts of the medullary plate withdrawn from them, with the result that the walls of the amnion have more or less collapsed, obliterating the cavity. In all probability the obliteration is an artifact, due to the rupture of the amniotic canals and the consequent escape of the amniotic fluid.

In the central region the entoderm has undergone a transformation to produce the notochord (n. ch.) which consists of a row of columnar cells. Already the entoderm shows signs of beginning to grow beneath the notochord, so that this structure will soon be cut off from the archenteron. The primordia of the pharyn-



geal pouches (*ph. p.*) are seen as bays of entoderm lying on each side of the neural tube.

The mesoderm in this region is in two rather distinct forms; the outer portion is epithelial in character and conforms to the general contour of the entire surface of the section; and the other part is composed of mesenchyme and lies to each side of the imperfectly formed brain vesicle, and consists of scattering stellate cells.

The medullary plate gradually grows narrower as one passes backward until the region of the somites is reached, where its width is about one-third that of the entire embryo. The margins of the entoderm have almost grown together beneath the notochord. The mesoblastic somites are partly constricted off from the lateral plates, which are undergoing the process of splitting into the somatic and splanchnic layers, between which is the weakly developed coelome.

In the region of the proximal part of the allantois (fig. 28) the belly-stalk bands are very much folded, having their outer margins turned up to form the scrolls that were noted in fig. 15. The umbilical blood vessels in the bands are well organized and are lined with an endothelium. The only other structure worthy of special mention is the posterior amniotic process which is reduced to a small flat tube.

The final section of this series to be considered here is one taken through the posterior end of the amnion (fig. 29). The amnion and median posterior portions of the belly-stalk bands are connected by a rather slender stalk with the Träger (*ms. co.*). The exact nature of the Träger will be considered in another section, and it remains here merely to point out that the original primitive knots are being rapidly transformed into villi.

The longitudinal section of the seven somite embryo (fig. 25) should be compared with that of the primitive streak stage, in order to bring out the most significant changes occurring in development. The notochord lies exposed throughout the greater part of its length, but at each end it is covered beneath with the entoderm. At the posterior end, where the notochord is covered over, the entoderm is seen to turn back on itself for a short dis-



tance (fig. 25, *en'*). This is doubtless only an expression of the same process noted in the study of cross section, in which it was seen that the entoderm was growing in beneath the notochord.

The primitive streak has become greatly reduced, due to its transformation into the embryo. The final change to which we would call attention is seen in the great reduction in the length of the allantoic entoderm (*al*). It is now not more than one-half of its former length, and is soon destined completely to disappear.

## V. HISTORY OF THE PLACENTA

Certain isolated stages in the development of the placenta have been described for at least three species of armadillo.

Kölliker ('76), Milne-Edwards ('78), and Duges ('79-'80), successively described the placental conditions seen in rather advanced vesicles of the South American nine-banded armadillo. Of these accounts that of Milne-Edwards appears to be the most detailed. The embryonic vesicle is described as being a pear-shaped body covered with a chorion, the proximal and distal parts of which were thin and membranous, while the middle part formed a thick, vascular, four-scalloped ring, composed of four fused placentae.

A stage similar to that just cited was recently described in somewhat greater detail by the present writers, ('09), and illustrated with two diagrammatic figures. This description of the North American variety of the species seems to agree closely with that of the South American variety as given by the authors just referred to. No doubt we have essentially the same species on both continents.

The only other reference to the placentation of *Tatu novemcinctum* is that of Lane ('09), who described in some detail the afterbirth of a specimen sent to him from central Texas.

A more comprehensive account of placental conditions is found for *Tatu hybridum*. Von Ihering states with reference to an advanced stage of placentation, that there is a zonary placenta which has nothing in common with that of the carnivora, but must be considered as a "*placenta annularis composita*." Each of the

eight discoid placentae is pressed against the margins of the two contiguous ones so that the whole set forms a ring or zone encircling the vesicle at right angles to the long axis of the uterus.

The most detailed account of the armadillo placenta yet published is that of Fernandez, who describes several important early stages of this structure in connection with his account of the early development of the *Mulita*.

Chapman ('01), gives a detailed description of the after-birth of a single specimen of *Dasypus sexcinctus*. Excellent figures of all structures involved accompany the text. As seen from the foetal side the placenta appears to be truly discoidal in form, but on the maternal side the distribution of the villi is decidedly different from that usually found on that type of placenta. The markedly arborescent villi are arranged in a broad, somewhat lobose ring around the margin of the disc, leaving the centre of the latter free of villi, a condition strongly reminding one of a much earlier stage in the development of the placenta of *Tatu novemcinctum*, when the original saucer-shaped *Träger* has begun to produce villi along the free overgrowing margin, but has a comparatively non-villous central area. The forked connection of the umbilicus with the placenta is almost identical with that found in our species. In view of these striking similarities in the placental details of the two species one is led to conjecture that the conditions found in six-banded armadillo closely approximate the ancestral conditions of the more highly specialized armadillos, of which *Tatu hybridum* seems to be the most pronounced example and *T. novemcinctum* the next.

In view of the fact that there has yet appeared no complete and consecutive account of the history of the placenta of any species of armadillo it seems worth while to devote a special chapter to a description of the conditions seen in our species.

For the earliest condition it will be necessary once more to call attention to the youngest embryonic vesicle of Fernandez. Here we find surrounding the true embryonic layers the trophoblast, which is attached to the uterine mucosa by means of a thickened disc or plug of trophoblast tissue, called the *Träger*. This attachment disc is to be considered as the primary placenta. As the

vesicle develops the Träger assumes a saucer-shaped form, as seen in vesicles 10 and 18 (figs. 12 and 14).

It will have been noted that, owing to the inversion of germ layers, the whole yolk-sac region of the vesicle is covered externally with entoderm, and that the trophoblast layer of this region, which in species with a diffuse placenta ultimately forms the outer lining of the villi, has practically disappeared. In the Träger region, however, the original trophoblastic epithelium persists in a somewhat modified form. This region of the vesicle consists of an inner layer of mesoderm, at this time rather thin and free of blood vessels, and an outer trophoblastic layer of true epithelial character, from the surface of which protrude branching and anastomosing cords of trophoblast tissue, which give to the Träger a characteristic rough or ridged appearance (fig. 12). These cords of cells appear to function at first as adhesive pads in that they no doubt serve to give the vesicle a firmer grip upon the uterine wall.

In the primitive streak stage these Träger cords, when examined histologically, show themselves to be composed of solid masses of cells with large nuclei and deeply staining cytoplasm, surrounded by a rather flattened layer of epithelium continuous with that covering the general surface of the Träger. Mitotic figures are of frequent occurrence among the cord cells, showing rapid cell proliferation. In some respects the appearance of the tissue suggest a glandular function, and it may well be that from it a secretion is given off which subsequently facilitates the penetration of the villi into the uterine mucosa. That these cords of cells are of trophoblastic origin seems certain, for the mesoderm, the only other layer in this region of the vesicle, is a thin membrane entirely separate from the trophoblast, which at this period it has not begun to invade. The Träger cords then must be formed by a process of rapid local cell proliferation which causes masses to protrude from the surface and frequently to overgrow it to such an extent that they appear to be almost completely constricted off (fig. 9).

Taking the primitive streak stage as the last phase of the primitive placentation, we may note that the Träger occupies roughly

one-third of the area of the embryonic vesicle (the remainder consisting of the yolk-sac region), that the embryos are attached to the Träger by paired bands of mesoderm, equivalent to the belly-stalk of the primates, and that the central area of the Träger is freer from thickenings than the periphery.

The function of the Träger or primary placenta appears to be not so much nutritive as merely adhesive, since there are at this time no blood-vessels in it by means of which nutriment might be conducted to the embryos. It is highly probable that whatever nutriment reaches the embryos comes to them by a process of osmosis through the thin wall of the yolk-sac region of the vesicle.

The formation of the secondary placenta occurs entirely within the confines of the Träger and involves at the beginning practically its whole area. A very instructive stage in the development of the placenta is seen in vesicle 18, (figs. 14 and 15). Here the Träger epithelium has been pushed out into short scaly villi, which show a tendency to overlap one another as well as the margin of the yolk sac region. These protuberances have been invaded by a stroma-like mesenchyme, which has arisen from the original thin mesodermal epithelium lining both Träger and yolk-sac regions of the vesicle. The free ends of the scale-like villi are tipped with masses of solid gland-like tissue derived by the breaking up of the branching cords of earlier stages into numerous knots which are carried out to the extremities of the individual villi. Although the general Träger epithelium which surrounds the villi has persisted in the form of a rather thick syncytial layer the knots are bare of covering except for the presence of an extremely thin layer of much flattened and scattered cells. The knot cells therefore are in a position to come into most intimate contact with the uterine tissues and probably serve as organs of penetration, softening the maternal tissues by means of a secretion and forcing open a path for the villi, in much the same way as the diamond tips of drills cut away the harder materials and open up a path for the shaft. These Träger knots forming the tips of the villi appear to persist throughout almost the entire foetal life in a form practically identical with that just described.

The tip of one of the branches of an arborescent villus is shown in fig. 11. The terminal knot of cells is seen to be practically naked, while farther down in the villus are shown blood vessels containing nucleated blood cells.

Although the formation of villi occurs at first over almost the entire area of the Träger, somewhat more advanced stages clearly show the beginning of a tendency for them to become restricted into four distinct patches near the boundary line between the Träger and yolk-sac and around the umbilicus of each embryo. The villi of other regions cease to grow and remain short, as in fig. 3, even flattening down into small rounded prominences which probably serve no nutritive function. Small patches of these flattened villi are scattered over the central area of the Träger as well as between the newly formed placental discs of the various embryos.

During this period the Träger area of the vesicle has been growing more rapidly than the yolk-sac region, the boundary between the two remaining at all times definitely marked. In fig. 3 is shown semidiagrammatically the conditions in vesicle 11 in which four discoid placentae are clearly marked off from the surrounding areas of scattering flat villi. At this stage the placentation is obviously discoid for each embryo.

In vesicle 14, (fig. 4) a decided change is in evidence. The four formerly quite separate discs have undergone a considerable increase in diameter and have come into very intimate contact along contiguous margins. This fusion is more complete between the placentae of embryos I and II and between III and IV than between II and III or I and IV. The significance of this is discussed later. A further change is seen in that the villous margin of the Träger region has overgrown the yolk-sac region (not fusing at this time with the latter) and has extended the placental area of the vesicle along the sides of the cervix cavity as far as the os uteri. Judging by the size and abundance of the arborescent villi in this placental annex it seems obvious that it plays the principal nutritive rôle at this period. One might compare this overgrowing fringe of branching villi to the cricoid placenta of *Dasypus sexcinctus*.

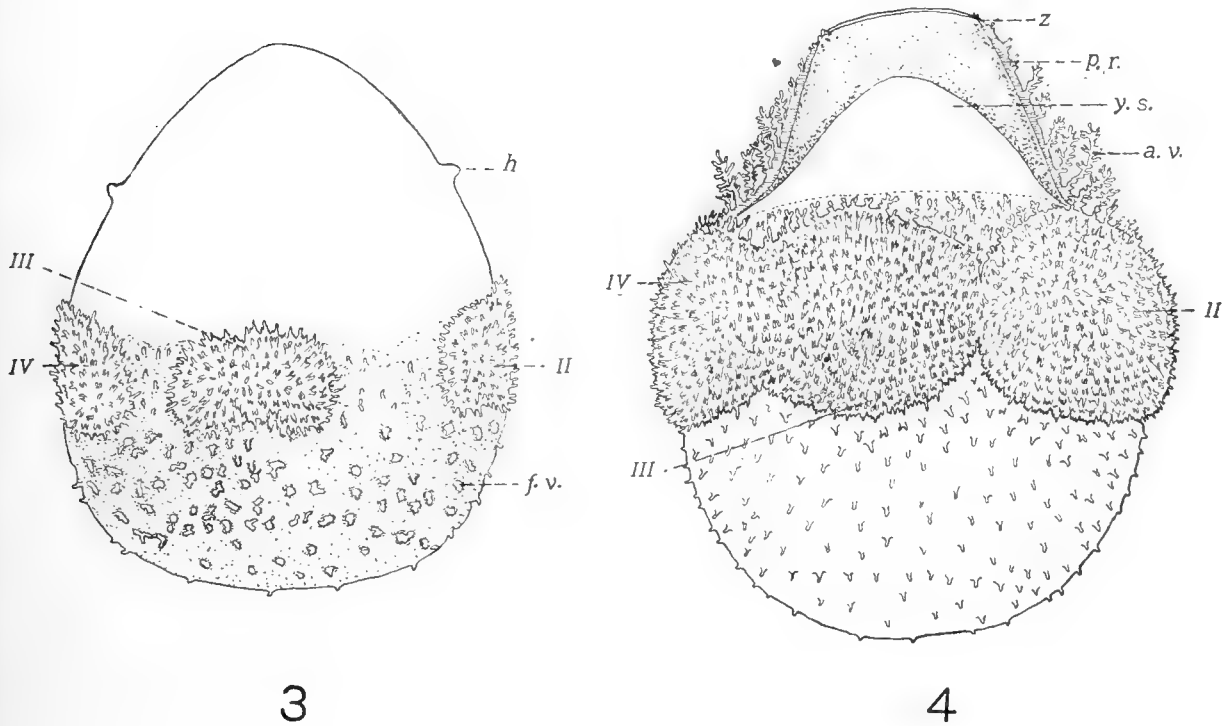


FIG. 3. A semi-diagrammatic representation of a vesicle seen from the dorsal side. II, III, and IV are the placental discs of the embryos so numbered. Note that those belonging to the paired embryos III and IV are closer together than II and III. *f.v.*, flattened villi of the Träger; *h.*, horn of the yolk sac.  $\times 2$ .

FIG. 4. A semi-diagrammatic drawing of the dorsal view of a vesicle slightly older than that seen in fig. 3. This shows the fusion of the placental discs I, II, III, and IV into a zone. Note that the fusion between the discs of III and IV (of paired embryos) is more intimate than between II and III. In the cervix region of the vesicle the dorsal part of the overgrowing placental ring, *p.r.*, has been removed to show the smooth yolk-sac lying within (*y.s.*). The ring was fused with the wall of the cervix at "z". The dotted line lying just above the discs represents the line along which the upper part of the ring was cut.  $\times 2$ .

The yolk-sac region of the vesicle is from this period on cut off from all contact with the uterine wall except at the mouth of the uterus where a small circular area remains uncovered by any outer layer. This condition persists until birth except that the overgrowing ring of arborescent villi undergoes a gradual degeneration, as the placental discs increase in functional prominence until the long, branched villi become mere flattened prominences, which serve only to slightly roughen the membraneous area at the cervix end of the vesicle.

The fundus end of the vesicle is still villous to some extent, but the villi are so small and scattered as to interfere only slightly with the transparency of the membrane. One can readily view the embryos in situ through this end. Subsequently the villi of this region disappear entirely with the exception of occasional small tufts that might readily be overlooked. In several vesicles (nos. 116 and 117) this region was seen to be four-lobed owing to the presence of two thickened bands of tissue crossing each other at right angles (figs. 37). These may indicate a demarkation of the several embryonic primordia earlier than that seen in the differentiation of the embryos themselves.

Stages intermediate between that shown in fig. 4 and the definitive condition can best be shown by a series of photographs.

Fig. 34 shows a somewhat older vesicle, in which the area at the fundus end is seen to be smooth and almost free of villi. The lobing of the composite zonary placenta is only slightly marked.

In fig. 35 is shown the cervix end of a stage slightly more advanced than the preceding one. The heavy coating of arborescent villi is seen to cover the entire cervix end of the vesicle with the exception of the small area that lies across the mouth of the uterus.

The dorsal surface of another vesicle, approximately of the same age as the last, is seen in fig. 36. The vesicle is attached to the shrunk cervix of the uterus. Here is evidenced the tendency on the part of the composite zonary placenta to divide into two double lateral discs. The deep notch occurs between the placental areas of embryos II and III. The small lobe (*d. b.*) is destined to persist as a bridge between the two lateral discs.

Two farther steps in the development of the definitive placenta are seen in figs. 38 and 39. The vesicle has grown to be several times the size of that shown in fig. 34. Coincident with this great increase in surface the villi in the composite zone have increased in functional importance while those that previously overgrew the yolk-sac region of the vesicle have degenerated, leaving a membranous area at the cervix pole, which in time becomes as large or even larger than that at the other end of the vesicle.



In fig. 40 is seen a condition slightly more advanced than that described in detail in our preliminary paper. There is now at each pole of the oval vesicle a star-shaped clear area, with a broad, deeply notched placental zone between, which still shows distinct signs of its origin from four discoid placentae. The notches are more deeply cut along the dorsal and ventral lines than along the lateral, where the placentae of the paired embryos I and II and likewise III and IV are so intimately fused as barely to show the points of union.

Shortly after the condition just described the placenta takes on what appears to be approximately the definitive condition. The tendency to form two well defined lateral discs is carried still farther, but in no case have we observed the complete separation of the two placental areas. As a rule the bridge between the two main discs is narrower on the dorsal side than on the ventral, but its narrowness is compensated for by the presence of a heavier coating of villi and by that of rather large placental blood-vessels which serve to connect one main disc with the other. It seems to be almost invariably the case that the division into the two double lateral discs strikes only approximately along the boundary lines of the original discoid areas, for colored injections forced into the placental vessels of individual foetuses run across the narrow placental bridges and invade more or less extensive and clearly marked villous areas of the other main disc. Such a condition is well shown in fig. 41.

The umbilical cords which may be from 18 to 20 centimeters long are attached rather near the fundus margin of the placentae except in rare cases where five foetuses occur and involve the crowding of one or more umbilical cords away from the margin.

Although a litter of young armadillos was born in the laboratory we were not fortunate enough to secure the after-birth and therefore cannot describe this final stage of the placenta. A comparison of the size and degree of development of the new-born young with the oldest foetuses in our possession convinces us that the conditions just described stand as definitive. Yet Lane in his reconstruction of the after-birth of the single individual under observation fails to find connecting bridges between the main discs. He



may have observed a rare case in which the line of separation into lateral discs passes exactly between the placental areas of the two dorsal and the two ventral embryos. Moreover we find no such clearly marked non-villous areas at the two poles as he describes. The smooth area at the cervix end is in all of our specimens very small and circular in outline, while that at the fundus end is only vaguely outlined and frequently shows patches of flat villi.

Any attempt to classify a placenta with the above history meets with grave difficulties, as one might conjecture from the multiplicity of terms applied to it by different writers. Kölliker in his original description of the conditions of the embryonic membranes of *T. novemcinctum* refers to the placenta as discoidal and deciduate. Milne-Edwards considers it to be compound zonary in structure. Beddard describes it as dome-shaped and deciduate; while Lane suggests the term "zono-discoidalis indistincta," subdividing Strahl's class "zono-discoidalis" into two varieties, "distincta and "indistincta."

Somewhat similar placental conditions, as found in *T. hybridum*, are designated by von Ihering as indications of a "placenta annularis composita." Chapman's use of the term "deciduate cricoid" appears to be apt for the placenta of the six-banded armadillo.

Of all these terms the one that appeals most strongly as descriptive of a certain rather persistent phase in the development of this multiformed structure is that used by von Ihering, "placenta annularis composita," but one must not forget that at first it is simply discoidal, then cricoid, then tetra-discoidal, later annularis composita, and finally incompletely doubly discoidal.

If animals are to be classified according to the form of their placentae, a method of classification that is fortunately falling into disrepute, it would be very difficult to classify the nine-banded armadillo, unless we arbitrarily decide to select some particular developmental phase of the placenta as a criterion for classification. In such cases one would be led to choose either the primary or the definitive condition and would thus call the placenta either "simply discoidal" or "incompletely doubly discoidal." Other terms scarcely find a rational basis.

The conjecture that the compound placenta of *T. novemcinctum* has been derived without any fusion of four embryonic vesicles from a condition similar to that described by Chapman for *Dasypus sexcinctus*, is very tempting in view of the evident close relationship of the two species and the striking resemblance that exists between them in the details of the placenta, umbilicus and other structures. This if true would furnish one of the most cogent proofs of polyembryony, since we find in the more highly specialized species a quadruple placenta, which at a rather early period closely resembles the definitive placenta of a more primitive species that gives birth to single young or to twins.<sup>3</sup>

## VI. HISTORY OF THE AMNION

From Fernandez's description of his earliest stage it is clear that the common amniotic cavity is at first the hollow of the ectodermic vesicle, which, through the inversion of germ layers, has come to lie within an envelope of entoderm. Regional differentiation of this ectodermic vesicle produces the ectodermal portions of the embryonic primordia, which are at first contained within a single vesicular amniotic cavity. Subsequently the individual embryos sink into pockets in the floor of the common amnion, which has evidently become fused to the walls of the yolk-sac at the cervix pole of the embryonic vesicle. The posterior end of each embryo has become fixed by means of the primordium of the belly-stalk to the margin of the Träger, and consequently, as the yolk sac gradually increases in size, the embryos are drawn away from the common amnion, retaining connection with it only by means of slender tubes, the amniotic connecting canals (figs. 12 and 14). It has been shown that each pair of embryos withdraws from the

<sup>3</sup> We are informed by Mr. Robert D. Carson, superintendent of the Philadelphia Zoölogical Garden, that a female six-banded armadillo in captivity gave birth to:

1. A single male, on May 10, 1901.
2. Twin males, on April 6, 1902.
3. Twins (male and female), on July 19, 1902.

common amnion into a single pocket and leaves for a short distance a single connecting canal. Later each member of these pairs loses its connection with its partner and acquires its own canal. This secondary separation of the pairs produces a forking of each of the original two connecting canals, a condition that persists for a long time.

After the embryos have left the common amnion the latter probably becomes functionless and ceases to grow. Fortunately however it persists with all of its connections through a considerable developmental period, furnishing evidences of polyembryony and of embryonic pairing. In fig. 44 it is shown still typical in form with its connecting canals entire but with their lumens interrupted with plugs of tissue. The regions between the plugs have become distended through local secretion of amniotic fluid, so that the canals as a whole present a decidedly moniliform appearance. In fig. 45 a somewhat more advanced stage of degeneration in these structures is seen. The common amnion can no longer be recognized but the canals are still clearly defined. Each of these shows a number of pronounced bead-like swellings, one of which may represent the remains of the common amnion. These canals may persist until stages as advanced as that shown in fig. 33, but are seldom to be detected in later stages.

The posterior amniotic processes, which in early stages were seen to be closely associated with the development of the allantois, do not persist in so marked a form in our species as in the *Mulita*. Only in rare cases does one see any traces of these structures at a period later than the five to seven somite condition (fig. 15). In vesicle 17, however, one of the embryonic amnia is connected by means of an amniotic canal with a sac as large or larger than the common amnion but lying at the opposite pole of the vesicle. This condition is no doubt exceptional and may be accounted for on the supposition that the posterior amniotic process of one of the embryos, on account of its unusual length, protruded far down into the *Träger* region, came into contact with and united with it, and subsequently swelled into an amniotic sac at the point where its terminal bulb fused with the *Träger* wall.

Another exceptional condition is that seen in fig. 46, where branching from a typical amniotic canal of one of the embryos, is an accessory canal running to an empty amniotic sac at the center of the Träger. Such a condition is doubtless due, as was stated in another place, to the presence of the remains of a degenerated fifth embryo. Teratological amniotic structures similar to those just described were observed in a number of other cases. In most instances there seems to be no doubt that they represent the retarded or degenerate remains of supernumerary embryos. The frequent occurrence of similar rudimentary embryos in *Tatu hybridum* and in our own species seems in itself a strong piece of internal evidence of specific polyembryony, for, on the basis of the origin of the several embryos from separate eggs, it would be difficult to understand why some should develop into complete embryos, and others, in the same vesicle and under practically identical conditions, should meet with so little success.

After the closure of the lumens of the various amniotic canals all communication between the four or more amnia is cut off; and henceforth each embryo has its own separate amnion in as true a sense as in those mammals that produce several entirely independent young. The developmental history of these envelopes is moreover in no important way different from that of other mammals except that in late stages a gradual fusion occurs, first of all with the wall of the chorionic vesicle and later with one another, where, through the pressure of growth their walls have come into contact.

Various representative stages in the later history of the amnion are seen in the photographs herewith presented. In fig. 44 the amnia may be seen to lie rather closely applied to the bodies of the embryos. In fig. 33 the cavities of the individual amnia have increased greatly in size and the sacs have assumed an ovoid form with the narrower end directed toward the cervix pole of the vesicle. In fig. 34, an external view of the fundus end of a somewhat older vesicle, the amnia are seen pressed against the membraneous area of the Träger, producing at points of contact an added transparency, reminding one of windows through which the embryos can clearly be viewed.

Even after the embryos have reached a length of 4 cm. the amniotic sacs are still quite free from one another, but a little later they begin to fuse along contiguous surfaces. Not until about a month before birth however do they become inseparably bound together. After the fusion is complete the amnia occupy the entire cavity of the vesicle and divide it into (normally) four quadrants of equal size, each running from pole to pole. This nearly definitive condition was described in detail in our preliminary account and needs no further attention here. In fig. 46 the edges of the amniotic partitions separating adjacent embryos may be seen at "a." The umbilical cords are always attached just to the left of the partitions.

## VII. HISTORY OF THE ALLANTOIS AND THE UMBILICUS

The early history of the allantois was shown to be very intimately bound up with that of the belly-stalk or primitive umbilicus. This intimate connection persists as long as the allantois retains a distinguishable structure. In stages of the degree of advancement shown in vesicle 17 and 11 (figs. 1 and 44) the entodermal allantois is seen as a slender cord of cells more or less closely fused with the umbilicus and showing here and there traces of a former lumen. The outlines of the mesodermal allantois, however, are no longer distinguishable from the tissues of the belly-stalk. The allantois of the armadillos seems then to be entirely vestigial in later stages of development.

The umbilicus arises directly from the primitive belly-stalk, which was shown in the description of vesicles 10 and 18 to consist of paired flat bands of mesoderm uniting the posterior end of the embryo to the margin of the Träger or primitive placenta. That the mesodermal allantois contributes some tissue to the definitive umbilicus has already been intimated, but at no time do allantoic blood vessels function. The placental circulation is carried on exclusively by the umbilical vessels, paired arteries and veins. Each artery arises along the inner margin of a belly-stalk band, while each vein forms in the scroll-like outer margin. In later stages the two bands fuse at a short distance from the vesicle

and continue to the body of the embryo as a single somewhat flattened cord. The forked connection between the cord and the vesicle is maintained as a characteristic feature of the placentation. In the definitive condition the umbilicus measures from 18 to 20 cm. in length and about 1 cm. in greatest diameter. The veins are longer than the arteries and take an open spiral course along the flattened edges of the cord.

### VIII. PAIRING OF THE EMBRYOS

In our preliminary paper attention was called, in treating of the nearly complete identity of the four embryos, to indications of a still closer resemblance between the individuals of the right and left hand pairs. In attempting to derive the four embryos from the blastomeres of the four-cell stage the following suggestion was offered: "This possible interpretation receives a striking confirmation in the fact that the four embryos can be arranged into two pairs, the individuals of which approach almost complete identity; and these identicals are not only adjacent to each other but are also attached to placental discs that are closely united. If all four embryos are derived from a single egg, this is exactly what we should expect to find; for surely the individuals derived from one of the blastomeres of the two-cell stage ought to be more nearly similar to each other than to the individuals of the other blastomere."

The subsequent acquisition of a large amount of additional data has served only to strengthen our conviction concerning this strong tendency toward pairing among the four embryos: a tendency that expresses itself in the method of separation of the embryos from the common amnion; in the fusion of the four discoid placental areas into two double lateral discs; in the different rates of development seen in the embryos of a single vesicle; and in the closer resemblance, as a rule, between the paired embryos of one double placental disc than between the embryos in general.

The forked arrangement of the amniotic canals, as was pointed out in connection with vesicles 10 and 18, shows that the embryos retreat from the common amnion in pairs and that only when at

some distance from the latter do the individuals of a pair sever their intimate connection and acquire separate amnia. Subsequently these embryos show their pairing in their mode of attachment to the definitive placental discs, embryos I and II being attached to the right hand disc and III and IV to the left.

Fernandez calls attention in the case of the *Mulita* to the exact identity in stage of development among the embryos of a set. That this is not always the case in our species is well brought out by a comparison of figs. 30 and 31, two embryos from vesicle 18.

Fig. 30 represents embryo III, and IV was identical with it. Fig. 31 was taken from embryo II but would serve equally well as a figure of I. The difference in degree of development between the two pairs is well marked not only in the number of somites (5 in III and IV and 7 in I and II), but in the conditions in the head region and in other parts.

It is not likely that a difference in rate of development between the two pairs is of common occurrence, but the clear case of it just presented seems worth recording not only on account of its rarity but because it serves to emphasize the tendency of the individuals of a pair to be alike, but somewhat different from the equally identical opposite pair.

Although of very common occurrence the pairing of embryos on the basis of resemblances in the total number of scutes in the nine bands of armor, is not without exception. In many cases the pairing is so marked as to be startling, as for example in one case where I and II each has 555 plates and III and IV each has 548; or in another case where I and II have respectively 551 and 552 and III and IV have respectively 560 and 559. In many other cases the pairing is obvious but not so clean cut.

There are on the other hand two cases where there was a close resemblance between three embryos, but one was strikingly different, as for example where II, III, IV have respectively 544, 545, 543 and I has 549; or again where I, II, III have respectively 562, 565, 564 and IV has 573. Finally two cases occurred in which, if any pairing at all exists it appears to be between I and III and between II and IV, as for example where I and III have respectively 544 and 546 while II and IV have 550 and 548.



On the whole however, in spite of these exceptions, the general rule holds good, that the closest resemblances occurs between paired embryos.

In this connection it should be mentioned that even where there is exact resemblance between the individuals of a pair in the total number of scutes in the nine bands of armor, there is no perfect correspondence with respect to individual rows. The resemblance in total numbers of scutes is however, a matter of more importance than the exact manner of their arrangement into rows, which is a secondary process. Each primary scute is the equivalent of a well defined hair group and these groups, as can be seen in other regions of the body, are quite definite units, although subject to more or less shifting before reaching their final arrangement into rows. In a subsequent paper we expect to make a special study of variation and heredity in the elements of the armor and shall in this place refrain from any more detailed reference to the subject.

Another source of data, however, which furnishes striking evidence of pairing is seen in connection with a fairly common tendency for regional fusion of adjacent bands of armor, or for the occurrence of interrupted and of incomplete bands in definite regions. Such atypical conditions occur in from three to four per cent of all cases, a fact that we have established from an examination of considerably over a thousand shells. This comparative rarity of occurrence, while it renders the collection of data on pairing and identity difficult, gives to such data an added value, in that chance resemblances are very unlikely to occur.

Only four cases of strikingly atypical armor arrangements have so far been discovered in the collection of fetuses now in our possession. In one case in embryos I and II there occurred a remarkably atypical scute arrangement in the first band of armor, while III and IV were quite normal. In a second case I and II showed a slight fusion between the first two rows at the right hand margin, while III and IV showed a much more extensive fusion in exactly the same region. The pairing in this case was only a matter of degree of fusion, but there was a decided difference in extent of the region of fusion in the two pairs. In a third



case III and IV exhibit almost precisely the same atypical condition, a short interruption in the first band a little to the left of the median line; II has an interruption in the same band, involving considerably more than half of the total length of the band, while I, although appearing to be perfectly normal, seems to have carried the tendency toward the suppression of a band to the extreme in that the whole band is lacking. In a fourth case one of the four embryos shows a short fusion between the first two rows on the left hand side, while the other three are perfectly normal.

Three out of four cases, then, furnish strong evidence of pairing, while the fourth case, which is after all atypical only to a minimum extent, affords an exception, whose weight can scarcely be sufficient to discredit the evidence of the other cases.

Although the pairing of embryos is not always perfectly obvious the cumulative evidence in favor of its general occurrence is convincing and must have some fundamental significance, an understanding of which is undoubtedly closely bound up with the early developmental mechanics as we shall attempt to show.

It has occurred to us that the division of the four-scalloped placental band into right and left lateral discs might be dependent upon the fact that the blood supply of the uterus comes from the paired ovarian blood vessels that enter the uterus laterally. It is true that the paired embryos, with very few exceptions are located on the same side of the uterus, but that the pairing is in any way causally related to the fact of their location near the entrance of a single maternal blood vessel is highly improbable, because the maternal blood does not reach the embryos.

It has also been suggested that the close resemblance between the individuals of a pair might be due to admixture of foetal blood, but we have demonstrated by the use of colored injections that the placental area of each embryo is sharply circumscribed and that no blood passes from one embryo to another. A common blood environment then cannot be held accountable for the near approach to identity seen in the pairs. Moreover it has been shown that long before there was any sign of the definitive placentation, and hence before there was any circulation of blood, pairing of embryos was evident in the relationship of the amniotic

connecting canals and in one case, in the degree of development of the embryos.

These observations force us to the conviction that the orientation of the vesicle in the uterus and the pairing of the embryos are expressions of the cleavage polarity and symmetry of the ovum. The cell products of the first two blastomeres would occupy the right and left halves of the early blastocyst and the daughter cells derived from the first two blastomeres would normally hold their relative positions as quadrants of such a blastocyst, so that, although it may not be possible to note any definite demarkation of embryonic primordia until a much later stage, they may be well defined from the first. When however pairing seems to exist between diagonally opposed embryos it might conceivably be due to a shifting of blastomeres in the four-cell stage, which could readily occur in such loose cell aggregates as prevail in early mammalian cleavage stages. A shifting upwards of two diagonally placed blastomeres and a consequent shifting downward of the other two would bring about a recombination of blastomeres into two new pairs without interfering with the hereditary tendencies of the individual units. Such an appeal to the imagination of the reader would scarcely be justified were it not the logical outcome of a failure to explain the conditions on any other basis. We are much inclined, in spite of Fernandez' failure to note any indication of a demarkation of separate embryonic areas in his earliest vesicles, to believe that such areas exist from the beginning and express themselves as separate primordia only on the differentiation of embryos. This view is in direct opposition to that of Fernandez who holds that up to the time when the separate embryos are distinguishable, the vesicle is a single embryo.

#### IX. CONDITIONS IN VESICLES CONTAINING FIVE FOETUSES

Out of a total of seventy embryonic vesicles there occurred three in which there were five foetuses. In all of these the sex could be determined and, curiously enough, they were all males. Whether or not this condition is universal could not be determined. If however it should prove that all five-embryo sets are males it

would mean that sex is determined by certain conditions in the egg. With only three cases in hand a discussion of the matter would be unprofitable.

In two cases out of three it was possible to enumerate the scutes in the nine bands of armor and on that basis to determine the varying degrees of resemblance among the embryos.

The occurrence of five embryos involves a decided asymmetry of the placental and amniotic elements and an atypical arrangement of the embryos. In each case the condition of two main lateral discs was maintained, but one of these discs, the one to which three embryos were attached, was considerably larger than the other. An examination of the larger disc shows that in each case it is composed of only two, not three, primary discs. One of the primary discs, on the side where three embryos are attached, is twice the normal size and to it are attached in symmetrical fashion the umbilical cords of two embryos. Apparently there is no regularity about the position of the double disc. In one case the double disc is ventral, and in the other two right lateral in position. Believing that the two embryos attached to a single primary disc are the equivalent of one typical embryo, we shall give them the same number, as for example, I and I'.

The following conditions are found in vesicle 91, the relative positions of the embryos being indicated in the diagram of the placenta, represented as cut open along the narrow dorsal bridge and laid out flat (fig. 5). The number of scutes in the nine bands of armor are indicated on the figure. It will be noted that there is distinct pairing on the normal side of the vesicle, between embryos III and IV; that the resemblance between the two embryos on the large disc (I and I') is equally close; but that there is a wide difference between these two embryos and the single embryo on the same side (no. II).

In vesicle 108 somewhat similar conditions exist, but the vesicle is laid open along the ventral bridge (fig. 6). Embryos II and II', having a common primary placental disc, are identical in the number of scutes but widely different from embryo I, which is attached to the other primary disc on the same side of the vesicle. Embryos III and IV are quite different from those on the other side, but are fairly similar to each other.

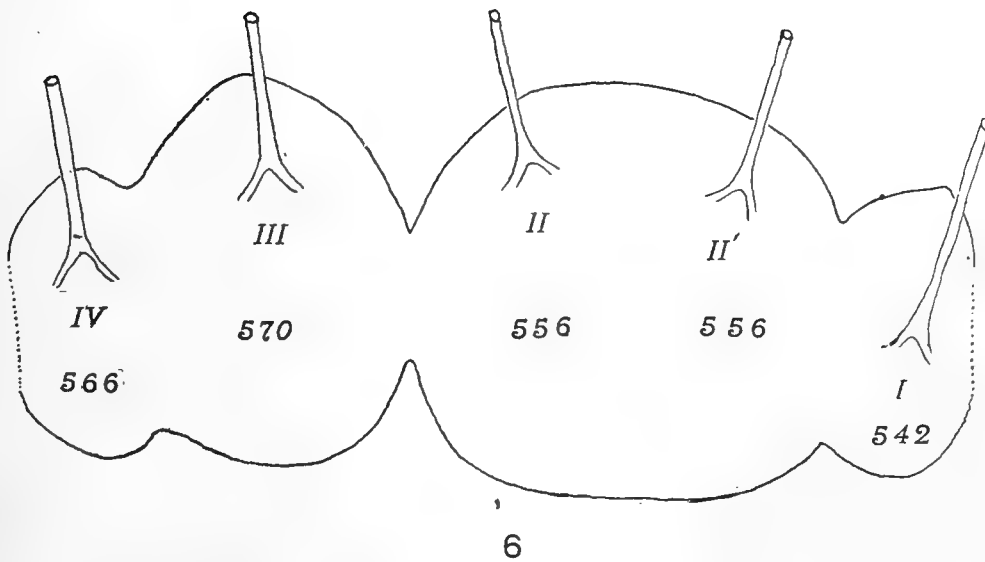
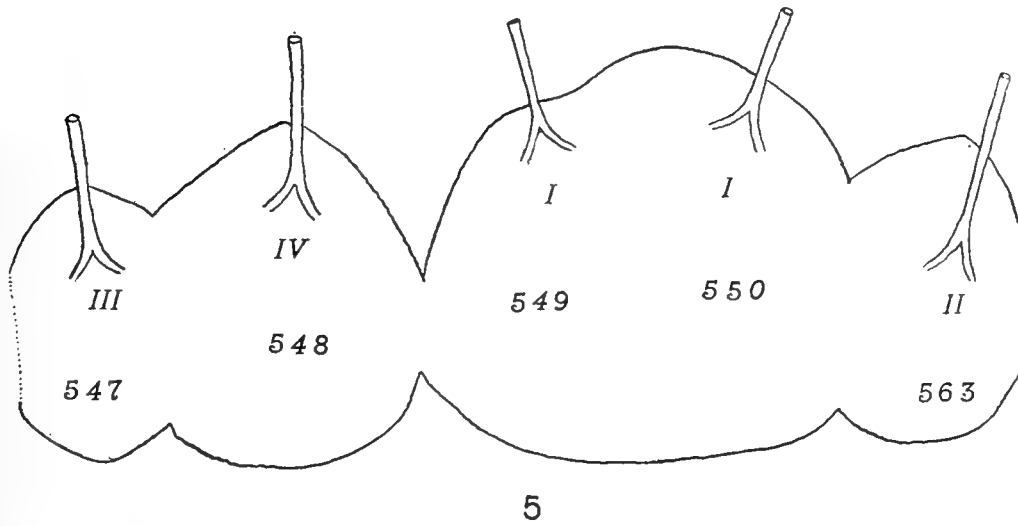


FIG. 5. Diagram of the placenta of vesicle no. 91, showing the placentation and the numbers of scutes in the nine bands of each embryo. Cut open along the dorsal notch.

FIG. 6. The same scheme for vesicle 108. Cut open along ventral notch.

In the case of the third five-embryo vesicle a satisfactory enumeration of the scutes was not found possible, but the position of the large disc was the same as in 108.

In all three cases the amnia of the three embryos occurring on the same side are irregularly arranged. Instead of occupying whole quadrants of the subspherical vesicle the amnion of one or more embryos is forced away from one end and crowded past the opposite end, thus causing the amniotic partitions to run diagonally across the placental discs instead of taking a meridional course from pole to pole as in typical cases. The relative positions of the embryos is of course correspondingly irregular so that one is immediately struck by it when the vesicle is first exposed to view.

The high degree of mal-adjustment seen in these vesicles would seem to indicate that the occurrence of more than four embryos is the expression of a coenogenetic tendency to carry polyembryony a step farther by a doubling of the present typical number of embryos. In the *Mulita* this condition has been attained and there exists a strong tendency to double again, as seen in the frequency of vesicles containing nine or more embryos. It appears probable to us in view of the occurrence of one case of twins in our collection, that in *T. novemcinctum* specific polyembryony had its origin in the acquisition of a habit of producing identical twins in a fashion similar to that seen in other mammals, that the inversion of germ layers made it easy for this tendency to express itself still more fully in the habitual production of four embryos. The production of more than four embryos in our species seems to involve so great a disturbance of a very accurate adjustment of embryos and embryonic membranes that it seems highly improbable that a larger number of embryos will ever become typical.

It would be interesting to find out whether there is in *T. hybridum* any tendency of the embryos to arrange themselves into two groups corresponding to the right and left sides of the vesicle. A study of Fernandez' photographs (figs. 1 and 2) would seem to indicate that such is the case. It is hoped that this matter will receive some attention and that the degree of resemblances among the embryos of the various sets will be determined.

## X. THE QUESTION OF IDENTITY OF EMBRYOS

In the case of identical or monochorial twins the question of close resemblance has been much discussed and the impression seems to prevail that the individuals of a pair show such marked similarity in their finer details of structure as to be practically identical.

In our earlier contribution to this subject we were inclined to look for the resemblances between the embryos of a litter and to underestimate the value of the points of difference. Now however that we feel that the question of specific polyembryony has been established, the differences among embryos interest us more than the resemblances, because they indicate a rather marked degree of versatility in the hereditary possibilities of a single fertilized germ cell.

The only point of unfailing identity among the individuals of a litter is that of sex. In 38 cases where the sex was definitely determined there was no exception to the rule that all embryos in a vesicle are of the same sex.

So far as dimensional differences go there is again practical identity, although in a few cases there seemed to be a slight difference in the size of the two pairs. In comparing one individual with another we were forced to admit that they differed only in the minutest details, such as the number of scutes in the armor. A comparison on this basis is just about as searching as would be a comparison of the number of feathers in a given feather tract of two birds, or of the hairs in a given hair area of two mammals. We have for the present limited our comparison to the total number of large scutes (with corresponding underlying bony plates), in the nine moveable bands of armor. The extreme range of variability in the total number of these plates (in all of the individuals so far examined) is rather wide, running from 511 to 620, a range of 109. In a number of cases the individuals of a litter exhibit a range of only five or six scutes, but as a rule the range is wider, averaging in all cases studied twelve, or less than one-ninth of the total range of our sample of the species. Whether or not this represents a closer resemblance than exists between

the individuals in a litter of rats or other mammals cannot at present be determined.

Although the difference between the two pairs of a litter may on the average be rather marked, that between the individuals of a pair seldom exceeds three scutes and averages in all cases observed less than three, while cases of absolute identity in the total number of scutes is of frequent occurrence.

It will be remembered also that in our discussion of pairing a considerable mass of evidence was adduced to show that even in atypical scute arrangements a high degree of identity existed between pairs, while in most cases the pairs differed greatly from each other. All of these observations go to show that the identity between the individuals of a pair is a very real thing but that there is nothing approaching true identity between the pairs. The condition may well be described as a case of double identical twins.

#### XI. SPECIFIC POLYEMBRYONY AND THE DETERMINATION OF SEX

The first clue to the existence of polyembryony in the armadillos was furnished by the discovery that all of the individuals of a litter are of the same sex. This together with his observation of a common chorion, led von Jhering to surmise that all of the embryos of a vesicle arise from a single fertilized egg. That this flash of insight foreshadowed the discovery of a truth has been sufficiently demonstrated, we believe, by Fernandez for *Tatu hybridum* and by us for *T. novemcinctum*.

Identity of sex then is in some way closely bound up with the phenomenon of polyembryony. Presumably all of the individuals of a litter are of the same sex because they have been derived from a single fertilized ovum; but this presumption involves the corollary that sex is determined in the germ before any demarkation of embryonic rudiments has occurred. The only alternative is that similarity of environmental conditions during the developmental period has the effect of producing offspring all of the same sex, an alternative with no factual basis, as is shown by the

following observations: that at a very early period each embryo is surrounded by its own amnion; that a little later each draws maternal nutriment from a separate area of the uterine wall; and that there is no admixture of foetal blood. We are therefore driven to the conclusion that sex is determined before there occurs any splitting of the single germ into separate embryonic primordia.

Opinions differ as to the exact period at which this splitting takes place. Fernandez maintains, on the basis of his studies of the early blastocyst of the mulita, that there is no trace of polyembryony until after the two primary germ layers have been laid down. What he probably means is that previous to this time there is no visible demarcation of the germ layers into isolated blastodermic areas. That the real separation of embryonic rudiments occurs at a much earlier period, even during the early cleavage stages (in our species at the four-cell stage), seems probable in view of the discovery of pairing among the embryos, a phenomenon for which no other explanation offers itself; and by the observations of Marchal, ('04), and Silvestri ('06), on the parasitic hymenoptera, where each embryo in a set takes its rise from a single cell of a rather advanced cleavage stage.

It seems highly probable then that the tissues involved in each of the four quadrants of an embryonic vesicle, whether or not they may show a demarcation, do really arise as the lineal descendants of one of the first four blastomeres. In this sense the four embryos are delimited at the four cell stage. It is hardly to be expected that any demarcation would be visible before the beginning of the period when the separate embryonic shields are differentiated.

The question as to the exact period of separation of the several embryonic rudiments is one that cannot at present be definitely settled. Even if one should be fortunate enough to obtain the early cleavage stages it is improbable that he would be able to observe any essential departure from the usual plan of mammalian cleavage, for a blastomere of the four cell stage would have the same appearance whether it were destined to produce a whole or only a quarter of an embryo.



It seems probable from our studies of the ovaries that the tendency to polyembryony is inherent in the unfertilized egg, which is the seat of a developmental vigor somewhat more intense than that exhibited in the ova of other mammals. This extra expresses itself sometimes by parthenogenetic divisions and at other times in the formation of fairly regular morulae within the confines of the Graafian follicles. That polyembryony is simply a more normal expression of the same superabundant energy in the female germ cells seems highly probable, and we would offer this as a tentative explanation of the physiology of polyembryony, pending an exhaustive study of a large collection of ovaries.

Taking it for granted then that sex is determined in the undivided oosperm, the question naturally arises as to which of the two germinal elements is the sex determiner. Cytological examination of the ovaries reveals no dimorphism of the ova. They all have 32 chromosomes and are equally alike in other respects. The possibility that sex might depend on which of the two ovaries produced the egg that became fertilized as suggested by the work of Dawson ('09). This writer maintains on observational grounds that in the human being the male producing ova come from the right and the female producing ova from the left ovary. The corpus luteum served to indicate which ovary functioned in any given pregnancy. In the armadillo we have an exceptional opportunity to put Dawson's theory to a test, for the corpus luteum of this species is a very prominent feature of the ovary that has functioned. A study of our data reveals the fact that the corpus luteum is found with almost equal frequency in right and left ovaries, which coincides with the exact equality of male and female litters. Unfortunately for the theory, however, there is no correlation between the sex of the embryos and the dextrality or sinistrality of the functional ovary. Out of twenty cases in which the right ovary contained the corpus luteum, the sex of the embryos was male in seven and female in thirteen; while out of thirteen cases in which the left ovary held the corpus luteum, the sex was male eight times and female five. Evidently then the position of the functional ovary has no determining influence on sex.

There is on the other hand excellent evidence that the male cell may act as a sex determiner. Studies of the spermatogenesis of our species show that the spermatogonial number of chromosomes is in all probability 31, one less than the oögonial. There is moreover in the reduction division a very definite and obvious odd chromosome, which precedes the other chromosomes to the pole of the spindle and serves to institute a dimorphism of the spermatids. That the odd chromosome is concerned with the determination of sex is as probable for the armadillo as for the insects and other forms in which it has been described. Both rest on the same observations. Since it is our intention to make a detailed study of the cytology of the germ cells in this species, it must suffice for the present to have indicated the sort of external evidence of polyembryony and of sex determination we have at our command.

The discovery of so clear a case of an accessory chromosome in a mammal is in itself worthy of mention, since it brings us a few steps nearer to the discovery of the physiology of sex determination in man. In addition to the intrinsic value of this discovery, however, we are afforded another strong proof of specific polyembryony, in that it is highly improbable, on the basis of the origin of the embryos of a vesicle from several fertilized eggs that each of these eggs would be fertilized by the same kind of spermatozoon. Such a possibility could be realized only through the instrumentality of selective fertilization, the occurrence of which has never been successfully demonstrated.

## XII. SUMMARY OF EVIDENCE FOR SPECIFIC POLYEMBRYONY

1. The uterus is simple, resembling that of the primates, which give birth typically to one offspring at a time.

2. There is never more than one large corpus luteum in the ovaries of a pregnant female.

3. In over 90 per cent of vesicles the number of normal embryos is four, a number that suggests their origin from the blastomeres of the four-cell stage. It is also unlikely that this number of ova would so often be given off at the same time.

4. The fact that all of the embryos of a set are invariably of the same sex strongly suggests their origin from a single fertilized egg.

5. The definite orientation of the embryos in the vesicle, and of the vesicle in the uterus, precludes the possibility of their origin from several eggs, even though these might conceivably be simultaneously given off from the ovary.

6. The inversion of germ layers presents a condition in both *Tatu hybridum* and in *T. novemcinctum*, which could not be attained by the union of several eggs to form a single vesicle. This is the strongest piece of evidence for specific polyembryony that has been advanced, and, to our minds, is practically conclusive.

7. The Träger or primitive placenta, common to all four embryos, is the morphological equivalent of that seen in the monembryonic vesicles of certain rodents.

8. The overgrowing fringe of arborescent villi seen in middle stages of gestation reminds one strongly of the cricoid placenta seen in the monembryonic vesicle of the six-banded armadillo, figured by Chapman.

9. The existence of partial or rudimentary embryos is evidence against the idea that the several embryos have been derived from separate eggs, for it is difficult to understand why some should develop perfectly, while others, under the same environmental conditions, should have so little success.

10. The pairing of embryos points to the origin of each pair from one of the first two blastomeres.

11. The presence of an accessory chromosome in the male germ cells suggests that the spermatozoon is the sex determiner. On this basis the fertilization of several eggs always by the same kind of spermatozoa seems highly improbable.

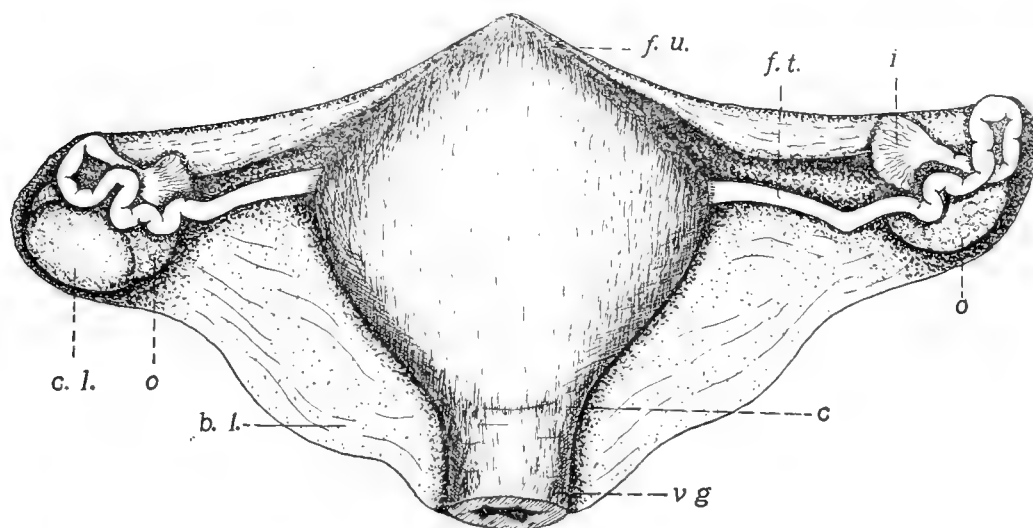
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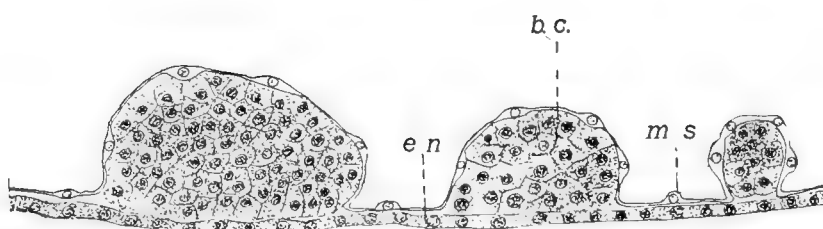
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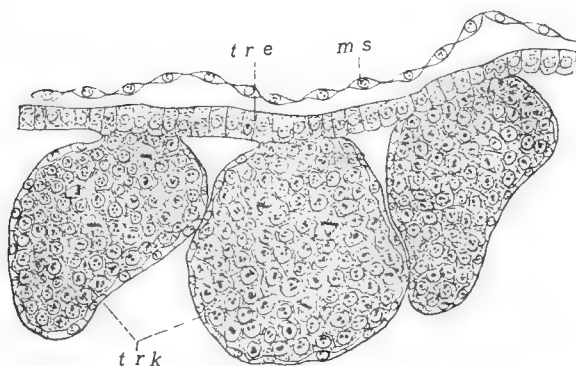
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|---|---|
| <i>a.a.</i> , amniotic attachment to wall of vesicle. | <i>i.l.</i> , intestinal loop.  |
| <i>al.</i> , allantois.                               | <i>l.s.</i> , lymph sinus.  |
| <i>al.en.</i> , allantoic entoderm.                   | <i>ms.</i> , mesoderm.  |
| <i>al.ms.</i> , allantoic mesoderm.                   | <i>ms.co.</i> , mesodermal connection.  |
| <i>am.</i> , amnion.                                  | <i>m.p.</i> , medullary plate.  |
| <i>am.c.</i> , amniotic cavity.                       | <i>n.ch.</i> , notochord.   |
| <i>am.c.c.</i> , amniotic connecting canal.           | <i>n.g.</i> , neural groove.  |
| <i>a.v.</i> , aborescent villi.                       | <i>n.l.l.</i> , notch of the left lateral lobe.   |
| <i>b.b.</i> , belly-stalk bands.                      | <i>o.</i> , ovary.  |
| <i>b.c.</i> , blood cords.                            | <i>p.am.</i> , posterior amniotic process.  |
| <i>b.s.</i> , belly-stalk.                            | <i>p.am.c.</i> , posterior amniotic cavity.   |
| <i>b.v.</i> , blood vessel.                           | <i>p.p.h.</i> , protochordal plate of Hubrecht.   |
| <i>c.</i> , cervix of uterus.                         | <i>p.p.</i> , primitive pit.  |
| <i>c.a.</i> , clear area of Träger.                   | <i>p.r.</i> , placental ring.   |
| <i>c.am.c.</i> , canal of the common amnion.          | <i>p.s.</i> , primitive streak.   |
| <i>c.e.</i> , canal enlargement.                      | <i>s.</i> , somite.   |
| <i>c.l.</i> , corpus luteum.                          | <i>s.am.c.c.</i> , supernumerary connecting canal of amnion.  |
| <i>co.</i> , cœlome.                                  | <i>s.t.</i> , sinus terminalis.   |
| <i>d.b.</i> , dorsal bridge.                          | <i>s.v.</i> , scale-like villi.   |
| <i>d.n.</i> , dorsal notch.                           | <i>t.m.p.</i> , tip of the medullary plate.   |
| <i>ec.</i> , entoderm.                                | <i>tr.</i> , Träger.  |
| <i>en.</i> , entoderm.                                | <i>tr.c.</i> , Träger cavity.   |
| <i>e.v.</i> , extra chorionic vesicle.                | <i>tr.e.</i> , Träger epithelium.   |
| <i>ex.c.</i> , extra embryonic body cavity.           | <i>tr.k.</i> , Träger knots.  |
| <i>f.g.</i> , fore-gut.                               | <i>u.m.</i> , uterine mucosa.   |
| <i>f.n.t.</i> , floor of the neural tube.             | <i>v.</i> , villi.  |
| <i>f.t.</i> , Fallopian tube.                         | <i>vg.</i> , vagina.  |
| <i>f.u.</i> , fundus end of uterus.                   | <i>y.s.</i> , yolk-sac.   |
| <i>f.v.</i> , flattened villi of Träger.              | <i>y.s.en.</i> , yolk-sac entoderm.   |
| <i>h.f.</i> , head fold.                              | <i>y.s.w.</i> , yolk-sac wall.  |
| <i>h.ms.</i> , head mesoderm.                         | I, II, III, and IV, refer respectively to the ventral, right lateral, dorsal, and left lateral embryos. |
| <i>h.p.</i> , head process.                           |   |
| <i>i.</i> , infundibulum of the Fallopian tube.       |   |



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FIG. 7. The genitalia of an adult virgin female as seen from the dorsal aspect. *b.l.*, broad ligament; *c.*, cervix of uterus; *c.l.*, coprus luteum in left ovary; *f.u.*, fundus end of uterus; *f.t.*, fallopian tube; *i.*, infundibulum; *o.*, ovary.  $\times 3$ .

FIG. 8. Portion of the yolk-sac wall in the region of the area vasculosa (see fig. 15). It shows three blood cords in section. These are made up of a central core of solidly packed cells, *b.c.*, which are surrounded by the mesodermal epithelium, *m.s.*  $\times 215$ .

FIG. 9. Cross section of the Träger of our youngest vesicle (fig. 12), showing three adjacent Träger cords or knots (*tr.k.*); *tr.e.*, Träger epithelium.  $\times 265$ .

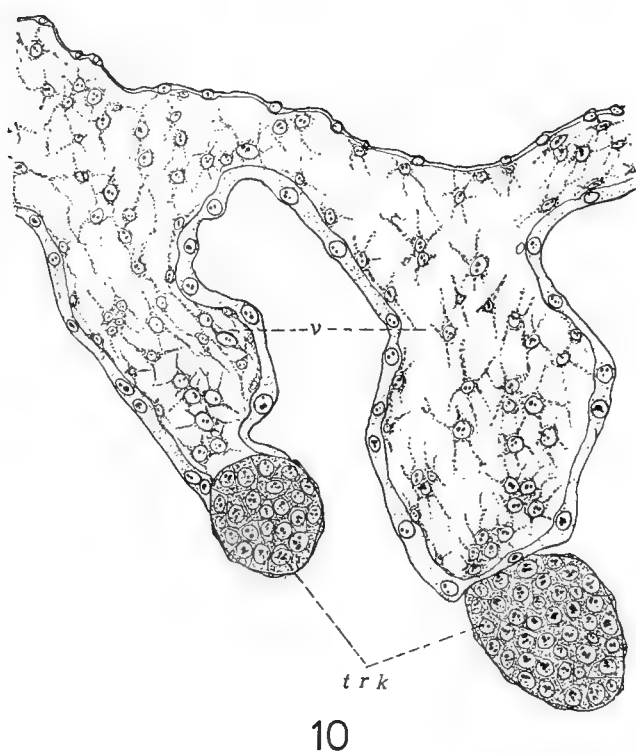
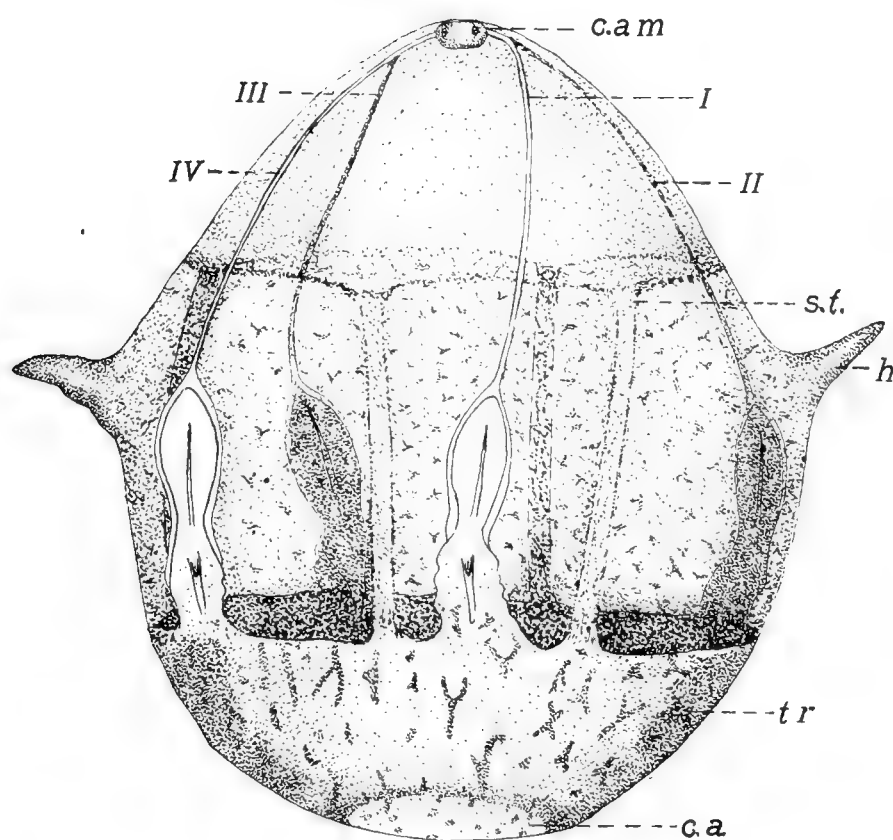


FIG. 10. Cross section of scale-like villi, *v.*, of the vesicle in fig. 14. The Träger knots are still covered with a thin epithelium. The epithelium of the villi has become a syncytium. The mesoderm has proliferated cells which have invaded the villi, but as yet blood formation has not taken place.  $\times 265$ .

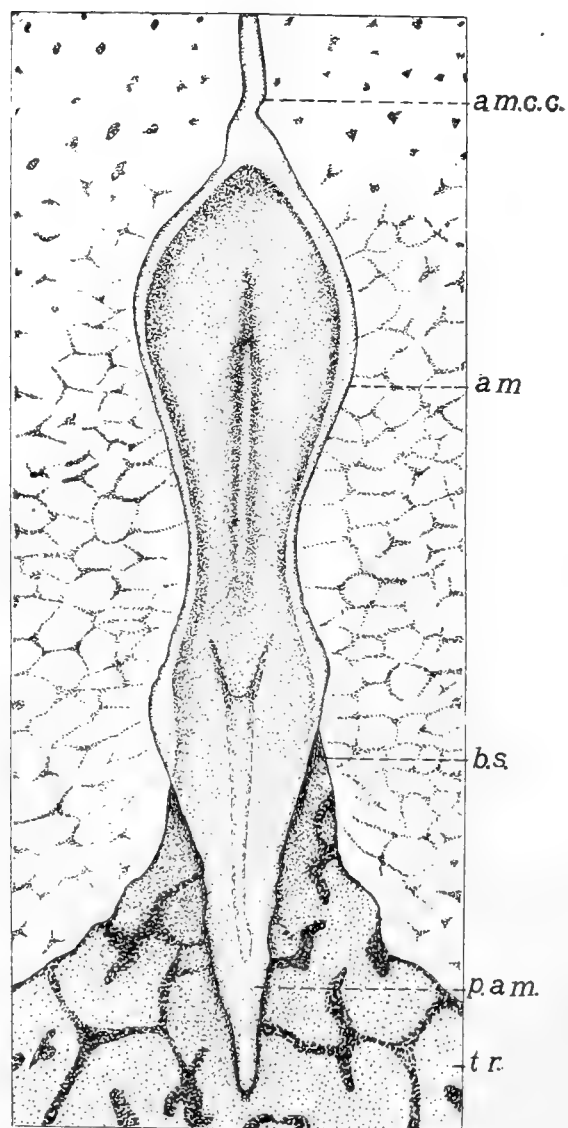
FIG. 11. The tip of a villus from a more advanced stage, showing, in addition to the features described in preceding figure, the well developed blood vessels, *b.v.*  $\times 265$ .



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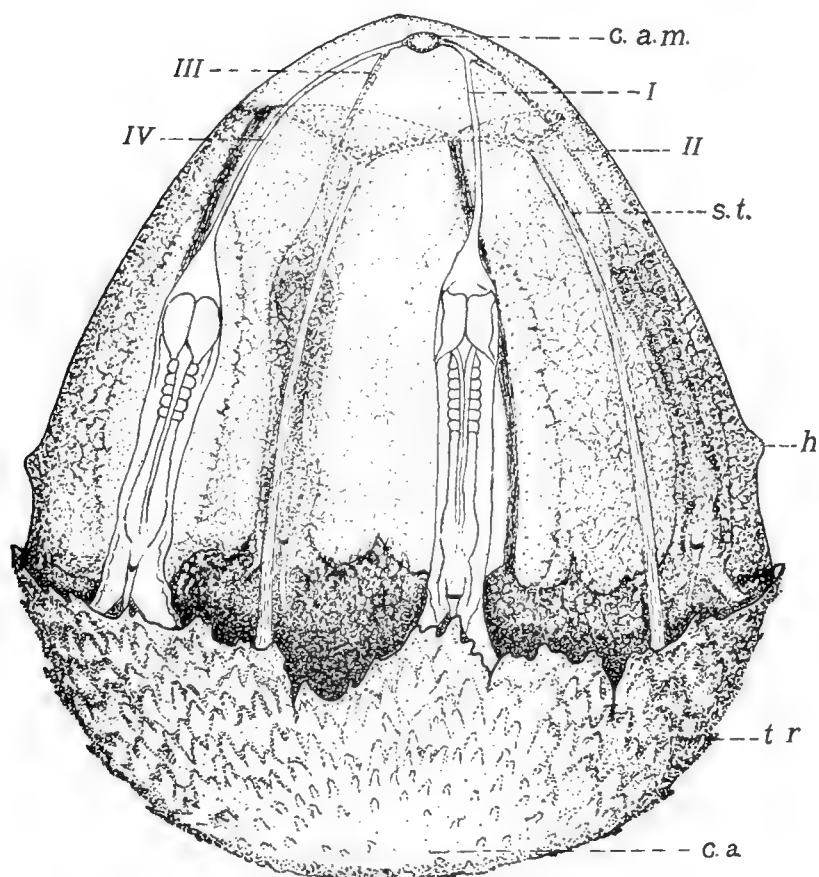
FIG. 12. A detailed drawing of vesicle no. 10 as seen from the ventral side as a semi-transparent object. The embryos in white (I and IV) are on the upper side of the vesicle, and since there is an inversion of germ layers, these are seen from their ventral aspects. Embryos II and III are shaded, and lie on the far side of the vesicle. For a fuller description see text.  $\times 9$ .





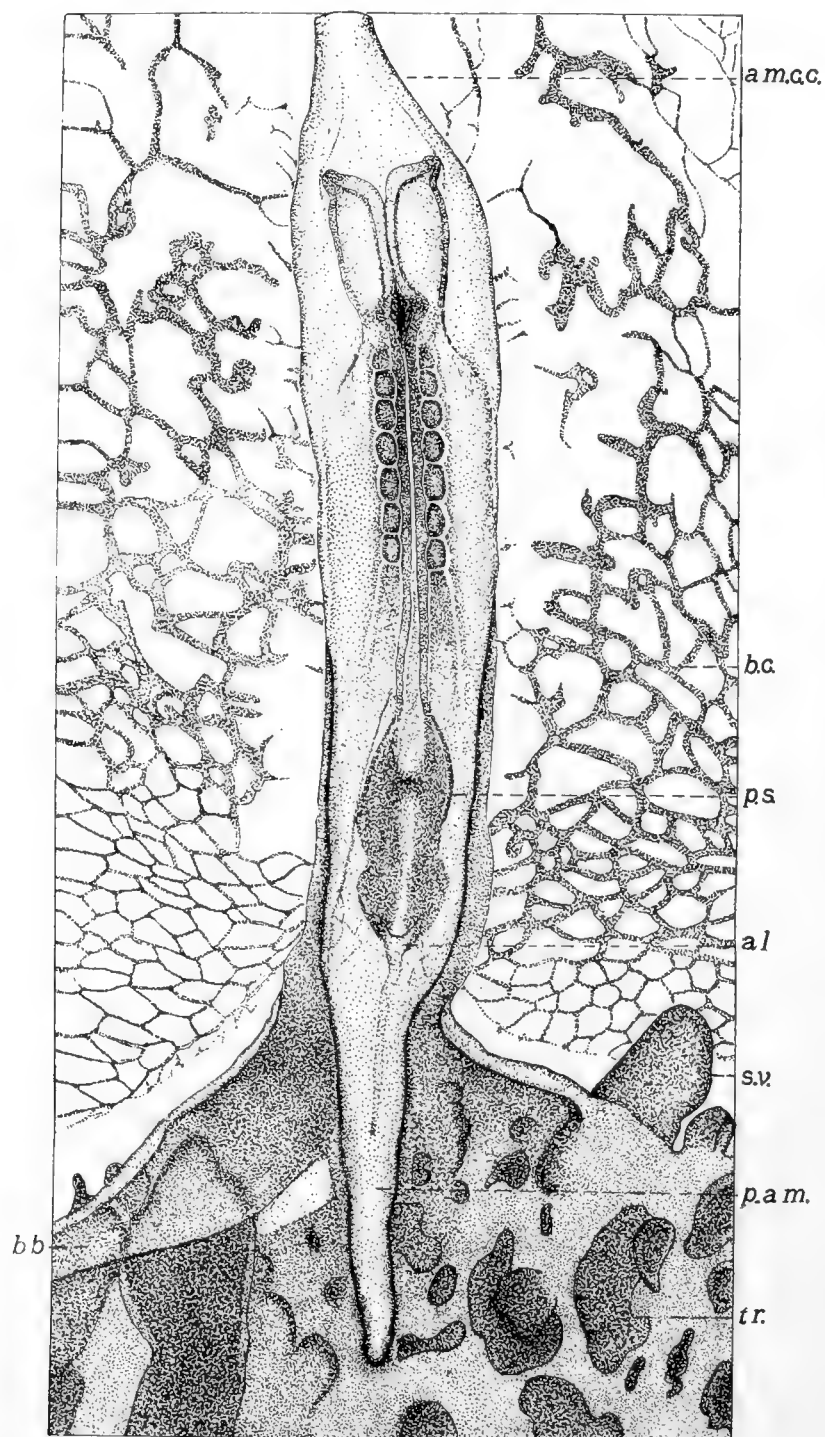
13

FIG. 13. A detailed drawing of embryo I (fig. 12) as seen from the dorsal aspect, that is, as viewed from the inside of the vesicle. *am.*, amnion; *a.mc.c.*, connecting canals of the amnion; *p.am.*, posterior amniotic process; *b.s.*, belly-stalk; *tr.*, Träger.  $\times 25$ .



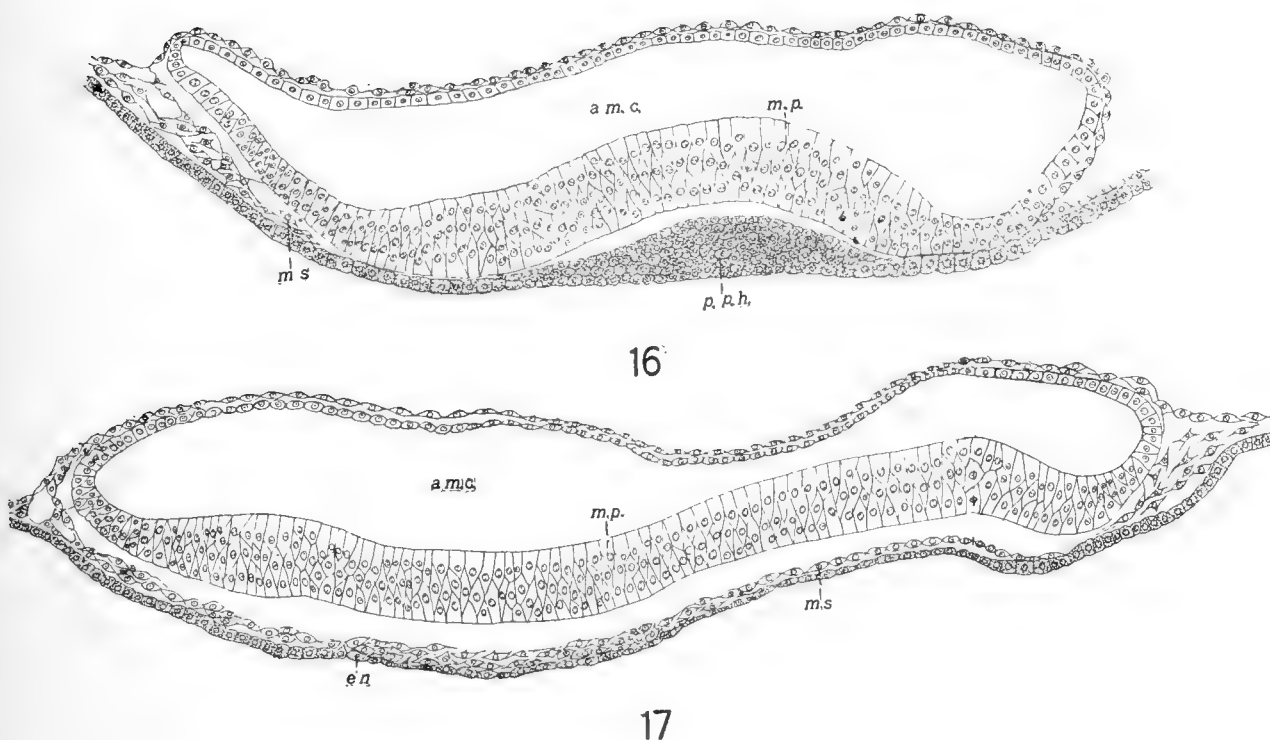
14

FIG. 14. Ventral view of vesicle No. 18, seen as a semitransparent object. The Träger is covered with scale like villi, which overlap the lower margin of the yolk-sac. This vesicle should be compared with that shown in fig. 12, in which the lettering is the same.  $\times 5$ .



## 15

FIG. 15. A detailed drawing of embryo I (fig. 14) as seen from the dorsal side. A photograph of this embryo is shown in fig. 31. *al.*, allantois; *am. c.c.*, connecting canal of amnion; *b.b.*, belly-stalk band, note that the band is much more distinct on the left side than on the right; *p.s.*, primitive streak; *s.v.*, scale-like villi; *tr.*, Träger region, which shows the villi as seen from their under sides. For a fuller description see text.  $\times 21$ .



NOTE—Figs. 16–23 represent a series of transverse sections taken through various regions of an embryo from the same vesicle as that shown in fig. 13.

FIG. 16. A section taken through the anterior end of the medullary plate. The most important feature of this section is the thickening of the entoderm to form the “protochordal plate” of Hubrecht, *p.p.h.*  $\times 130$ .

FIG. 17. A section taken through the medullary plates at a point lying half way between the fore end of the head process and the anterior tip of the embryo. The entoderm is distinct from the mesoderm, which is scarcely more than one cell thick.  $\times 130$

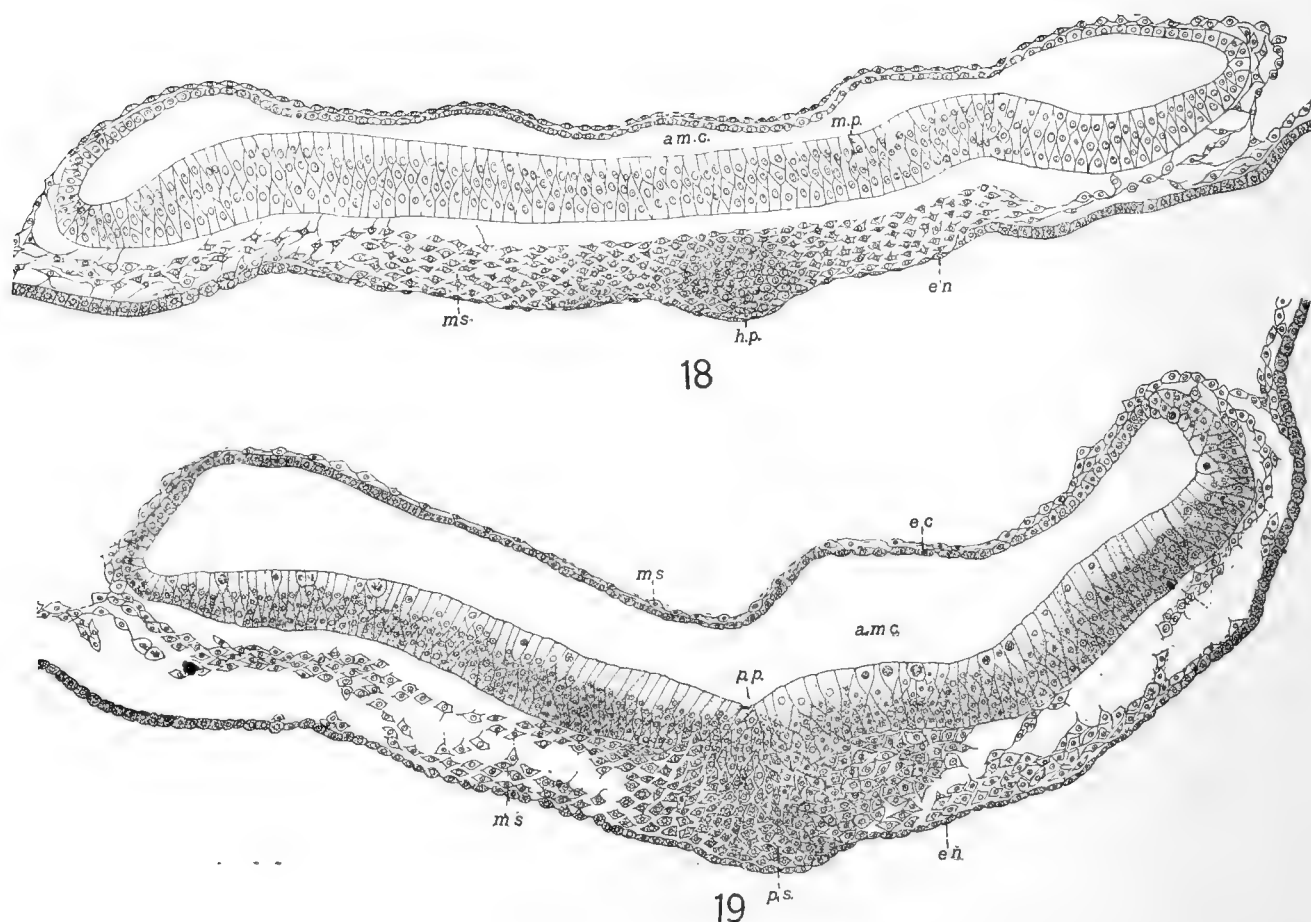


FIG. 18. A section taken through the middle of the head process. In this region the entoderm is very intimately associated with the mesoderm, especially in the central part of the section.  $\times 130$ .

FIG. 19. A section taken through the primitive pit. It shows the primitive streak proliferating mesoderm in the characteristic manner.  $\times 130$ .

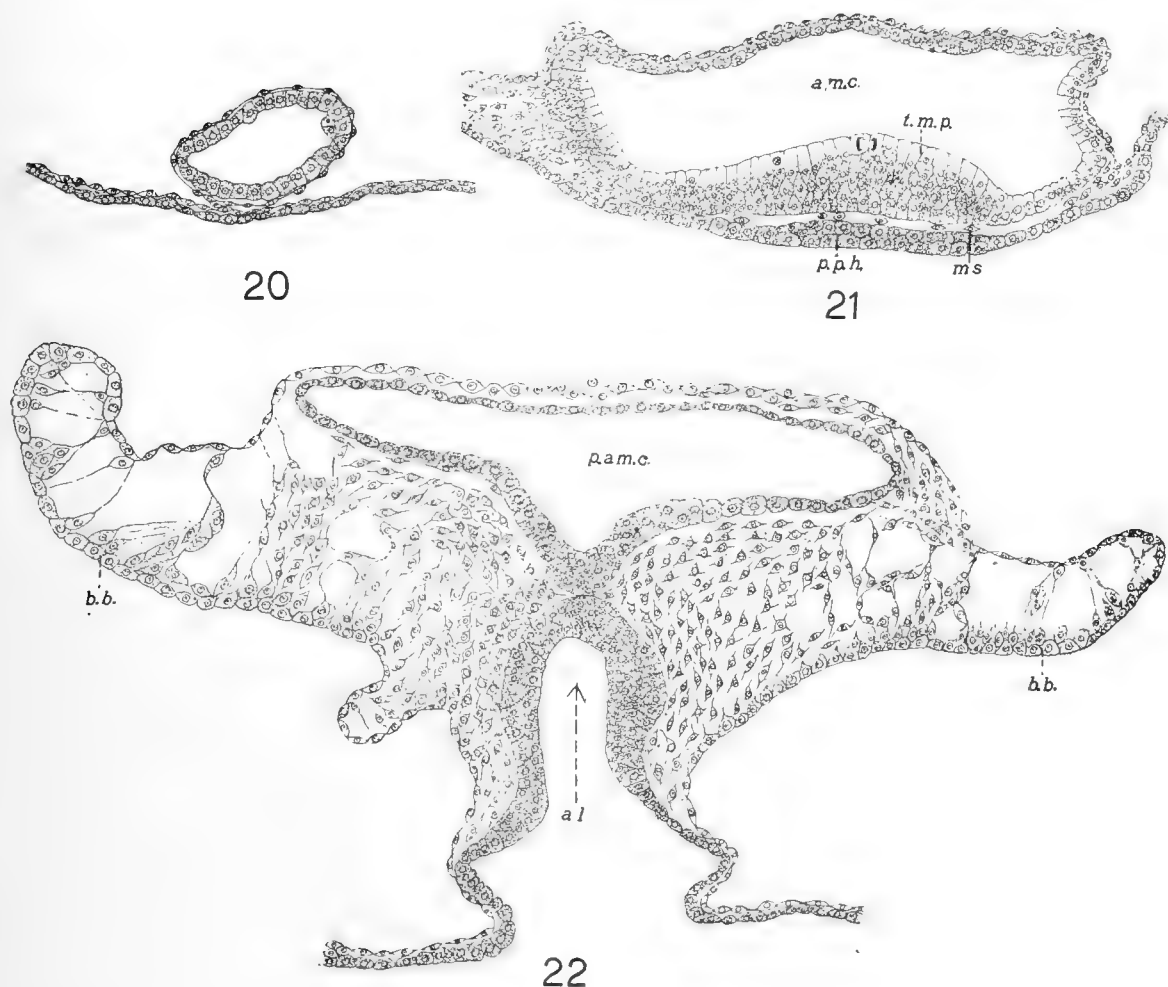


FIG. 20. A section taken through the connecting canal, which is seen to be composed of two layers, ectoderm on the inside and mesoderm on the outside, and is loosely connected with the mesoderm of the yolk-sac wall.  $\times 143$ .

FIG. 21. A section taken through the tip of the medullary plate. This is the first section that shows the anterior end of the protochordal plate of Hubrecht. Note that there are a few scattering mesoderm cells (*ms.*) that have wandered in between the plate and the ectoderm.  $\times 143$ .

FIG. 22. A section taken through the belly-stalk at the level of the mouth of the allantois (*al.*). The cavity of the posterior amnotic process (*p.am.c.*) does not cover more than one-half the width of the section. The mesoderm of the belly-stalk extends laterally to form wing-like processes. These are the belly-stalk bands (*b.b.*) through which the umbilical blood vessels pass to the Träger.  $\times 143$ .

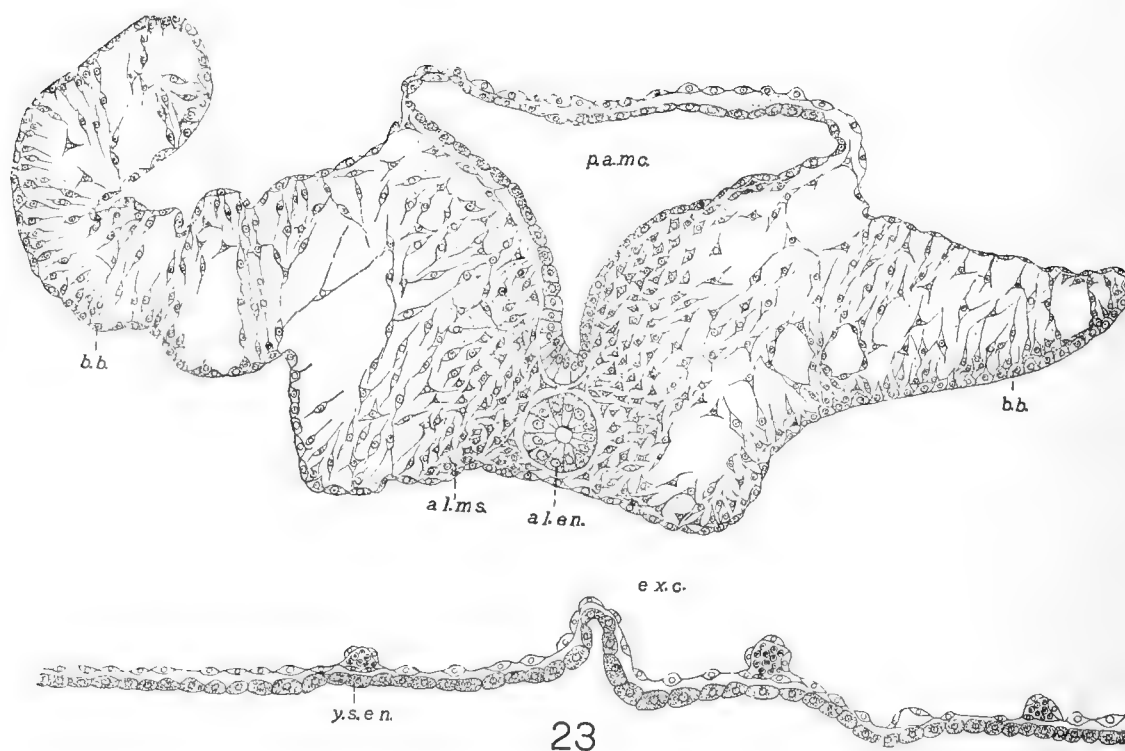
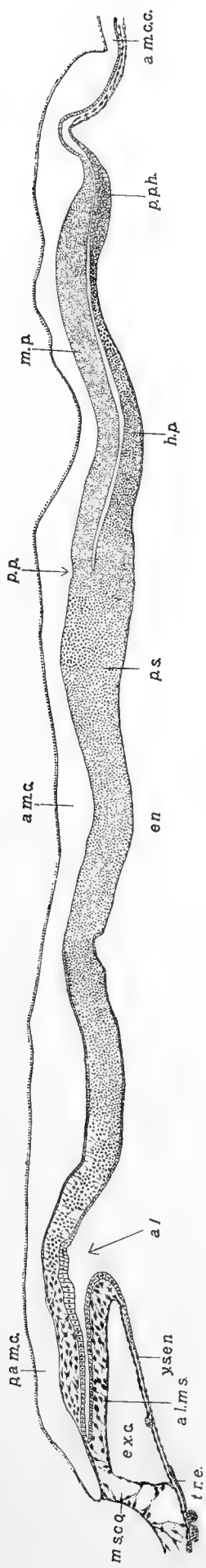
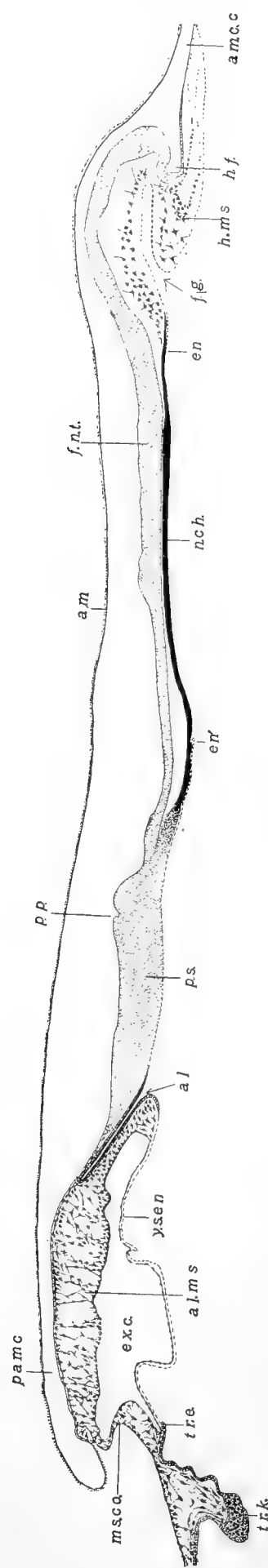


FIG. 23. A section taken through the belly-stalk near the posterior tip of the allantoic entoderm (*al. en.*). The mesoderm is indistinctly divided into two portions. (1) that forming the belly-stalk bands, and (2) that part immediately surrounding the allantoic tube—this may be called the allantoic mesoderm (*al.ms.*). The belly stalk is here separated from the wall of the yolk-sac by a space (*ex.c.*), which is only a part of the general extra-embryonic cavity.  $\times 143$ .



24



25

FIG. 24. A semi-diagrammatic longitudinal section of the primitive streak stage. *al.*, mouth of allantois; *ms.co.*, posterior mesodermal connection between the belly-stalk and the Träger; *p.p.h.*, protochordal plate of Hubrecht. See table of reference letters for further explanations.  $\times 47$ .

FIG. 25. A similar section for the seven somite stage. As compared with the preceding stage, the following are the principal changes that have taken place: (1) formation of the fore-gut, *f.g.*; (2) differentiation of the head mesoderm, *h.ms.*; (3) formation of the notochord, *n.ch.*; (4) reduction in the length of the allantoic entoderm, *al.*; and (5) formation of the head-fold, *h.f.* Attention should be called to the condition of the entoderm. It is wanting directly beneath the notochord, except for a short distance at each end. At the posterior end of the notochord the entoderm turns back on itself for a short distance (*en.*).  $\times 38$



## PLATE I

### EXPLANATION OF FIGURES

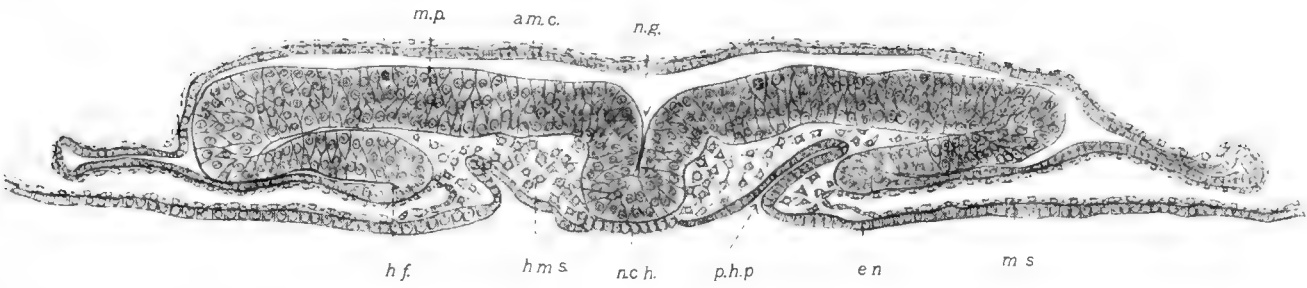
NOTE, figs. 26-29 represent a series of transverse sections of a five-somite embryo.

26. A section through the region of the head-fold. The brain vesicle is in the process of formation, and the neural groove (*n.g.*) has become very deep. The notochord (*n.ch.*) is represented by a row of cells, and to each side of it the entoderm is bayed to form the pharyngeal pouches (*ph.g.*).  $\times 68$ .

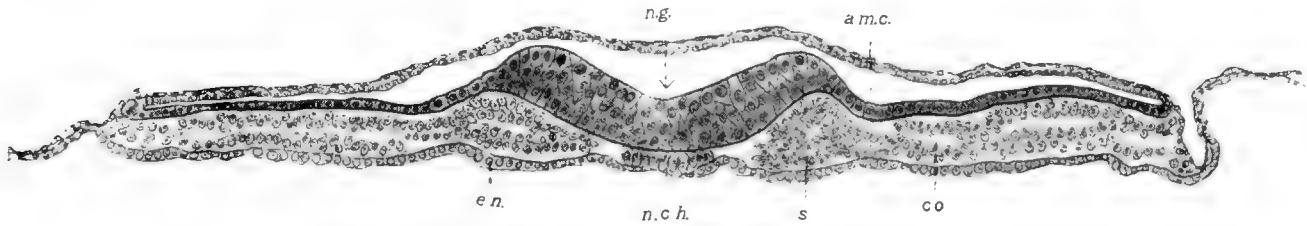
27. A section through the somite region. The somite shows a distinct cavity, and the coelomic cavity is forming. The entoderm is beginning to close in beneath the notochord.  $\times 68$ .

28. A section through the proximal part of the allantoic tube. The bands of the belly-stalk have become much folded, and contain a number of umbilical blood vessels. The posterior amniotic process has become reduced to a very small tube.  $\times 68$ .

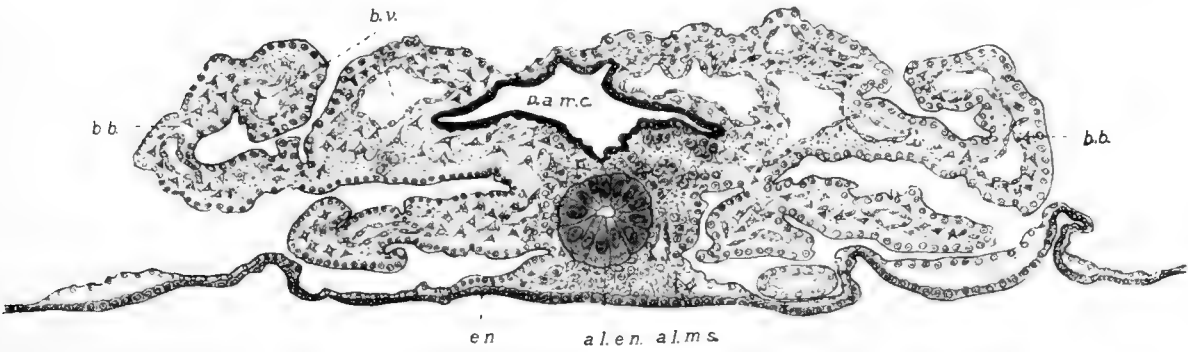
29. A section through the posterior mesodermal connection (*ms.co.*) of the belly-stalk. The Träger shows the villi in the process of formation.  $\times 30$ .



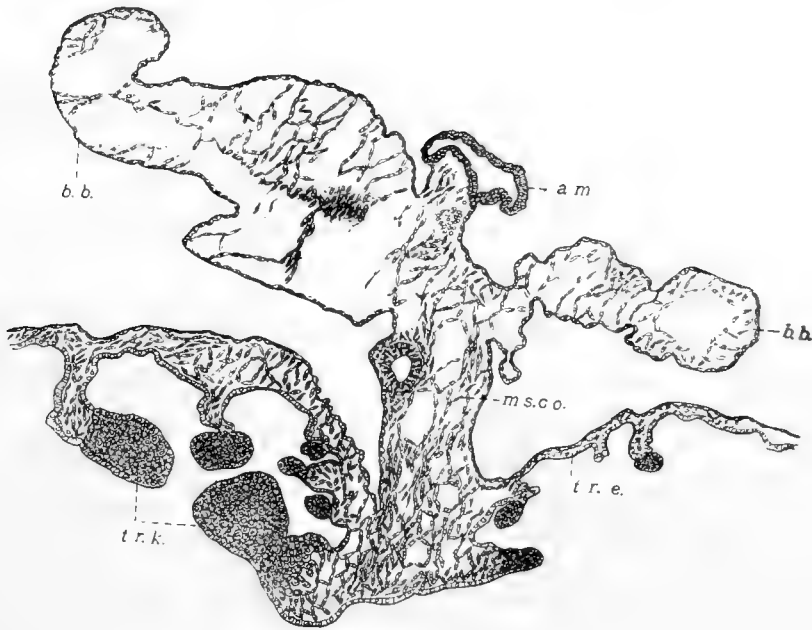
26



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29

## PLATE II

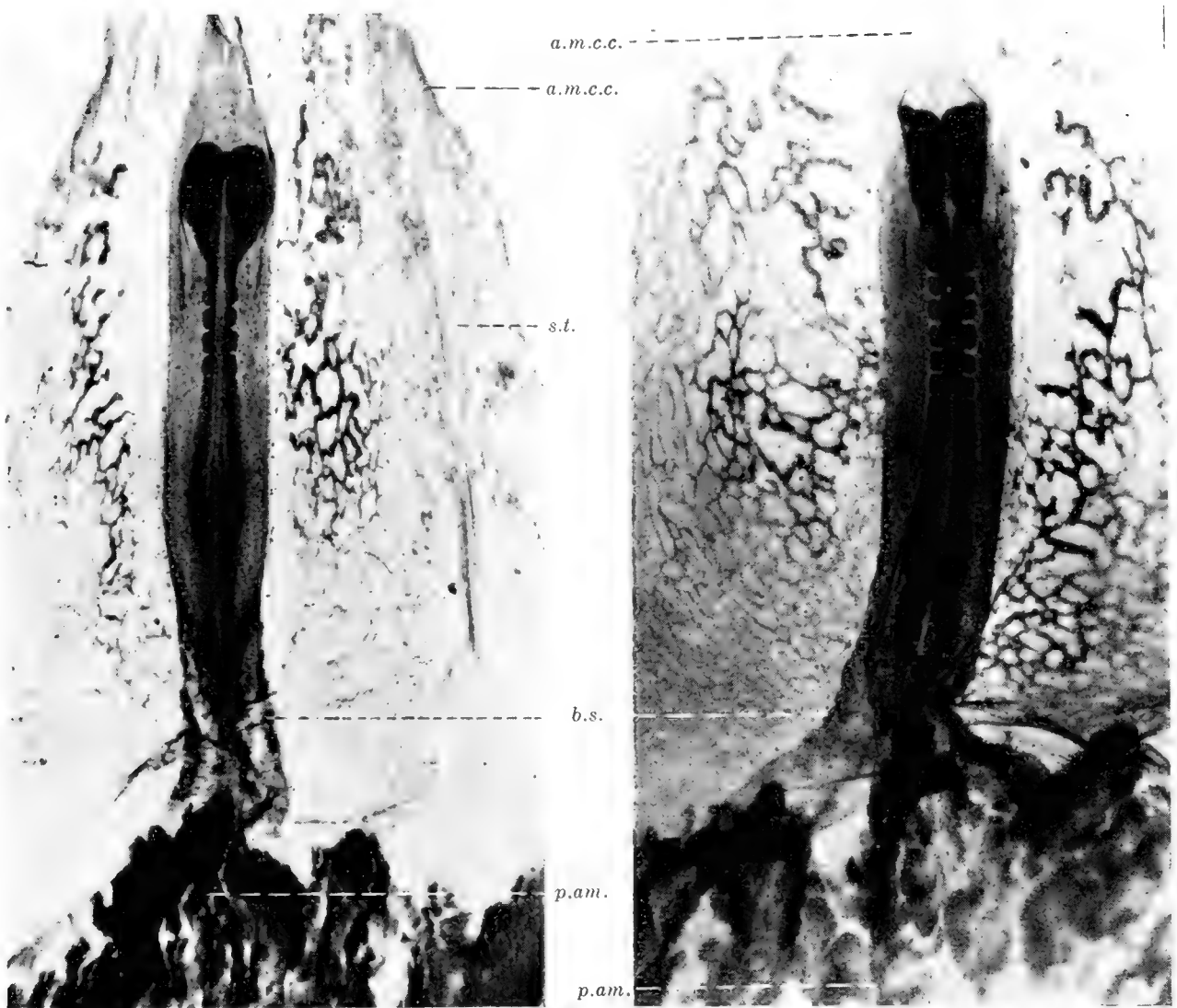
### EXPLANATION OF FIGURES

30. One of the five somite embryos (III) of vesicle No. 18 (see figs. 14 and 32). Note how the embryo is attached to the Träger by means of the belly-stalk (*b.s.*). The area vasculosa, like that of the chick, does not extend in to the embryo, but is separated from it by a clear space which corresponds to the area pellucida. On the right is seen the compound sinus terminalis (*s.t.*) lying between the vascular areas of the two contiguous embryos. The posterior prolongation of the amnion is not clearly seen, but its extreme tip is indicated by the leader, *p.am.*  $\times 16$ .

31. A seven somite embryo (I) of this same vesicle. For a description of this embryo see the detailed drawing shown in fig. 15.  $\times 16$ .

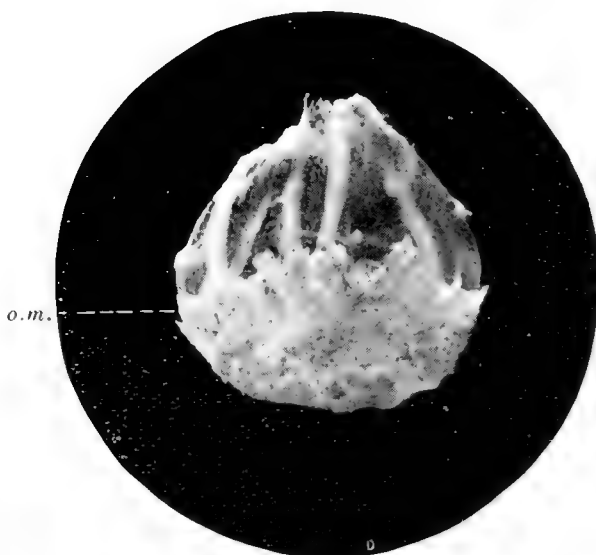
32. The dorsal view of the vesicle reconstructed in detail in fig. 14. The cervix end is slightly torn and is turned under, consequently the common amnion and its canals are not shown in the photograph. The turning under of the torn piece also makes the vesicle appear shorter than it really is. At *o.m.* may be seen the scale-like villi beginning to overgrow the lower portion of the yolk-sac.  $\times 2.15$ .

33. A vesicle cut open along the mid-ventral line and spread apart to show the pairing of the embryos. It will be noted that the embryos are arranged so that the right-hand pair (III and IV) is the mirrored image of the left-hand pair (I and II). At this stage the amnia are still distinct, and in shape are oval with the broad end directed toward the fundus.  $\times \frac{1}{2}$



30

31



32



33

## PLATE III

### EXPLANATION OF FIGURES

34. A view of the fundus end of a vesicle which contained embryos measuring 31 mm. head rump length.<sup>4</sup> In the portion of the vesicle lying within the margin of the placenta are seen four window-like spots. These are the areas where the amnion come in contact with the wall of the vesicle. The fundus end is now practically free of villi.  $\times \frac{1}{2}$

35. A view of the cervix end of a vesicle in which the embryos measured 31 mm. The clear yolk-sac is seen through the opening in the rather thick placental overgrowth. The margin of this opening represents the place where the placenta is attached to the uterine mucosa at the cervix end of the uterus.  $\times \frac{2}{3}$

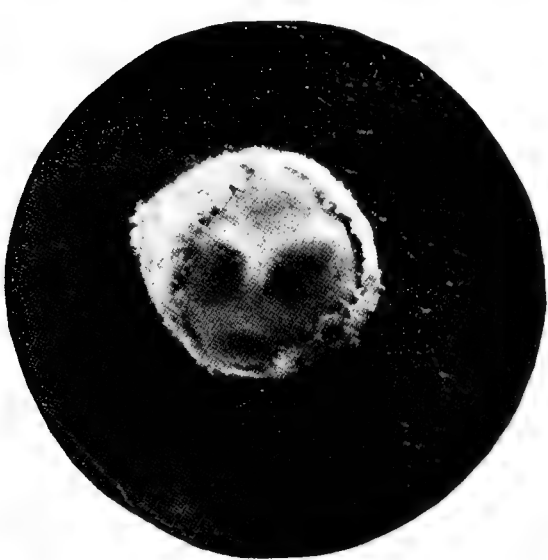
36. The dorsal view of a vesicle which is still attached to the cervix of the contracted uterus. This vesicle shows a distinct placental bridge (*p.b.*) connecting the lateral placentae, and also a number of blood vessels at the fundus end. Embryos 32 mm. in length.  $\times \frac{2}{3}$

37. A view of the fundus end of a vesicle which contained embryos measuring 33 mm. This view shows two points worthy of especial note: (1) the four-lobed appearance of the fundus membrane, due to constrictions occurring between the fundus areas of the individual embryos (seen more clearly before fixation); (2) the persistence of a few villi, which in the photograph appear as scattering black specks.  $\times \frac{2}{3}$

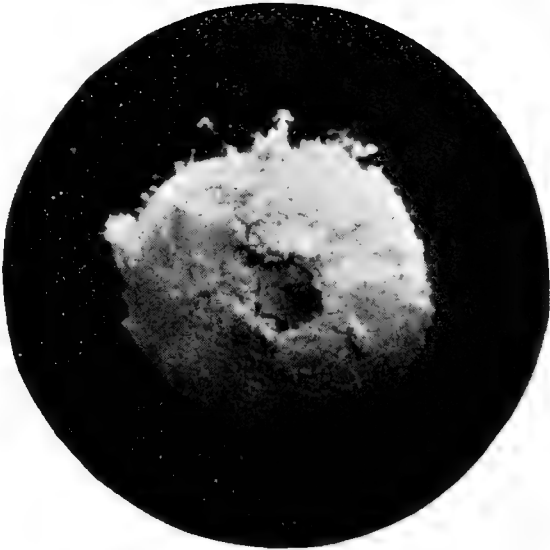
38. A view of the ventral side of vesicle, with embryos measuring 36 mm. The cervix end of the yolk-sac is clearly visible, and blood vessels are seen at the fundus end. The placental bridge although present is not clearly brought out in the photograph.  $\times \frac{2}{3}$

39. A view of the ventral side of a vesicle which contains embryos measuring 53 mm. The division of the zone-like placenta into right and left halves is clearly brought out. The fundus end of the vesicle is now practically free of both villi and blood vessels, and the membranous area at the cervix is much larger than in the preceding figure.  $\times \frac{2}{3}$

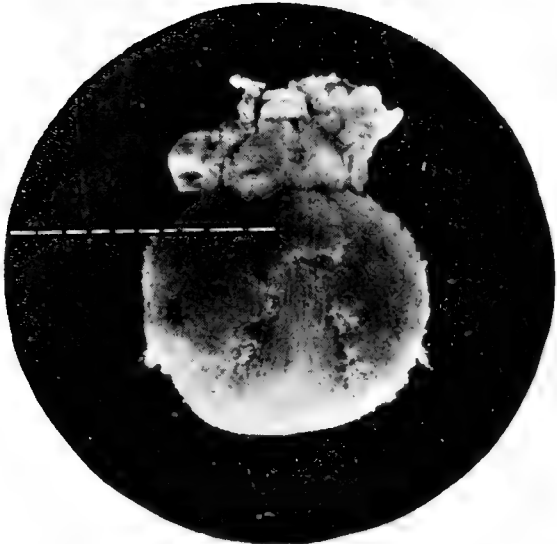
<sup>4</sup>Unless otherwise stated, the length of the embryo will mean the head-rump measurement.



34



35



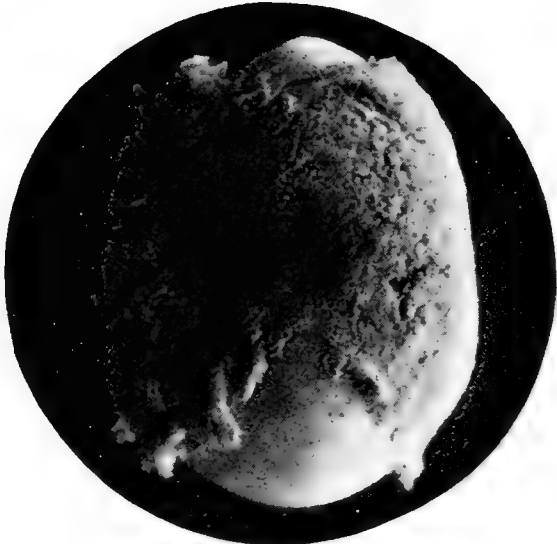
36



37



38



39

## PLATE IV

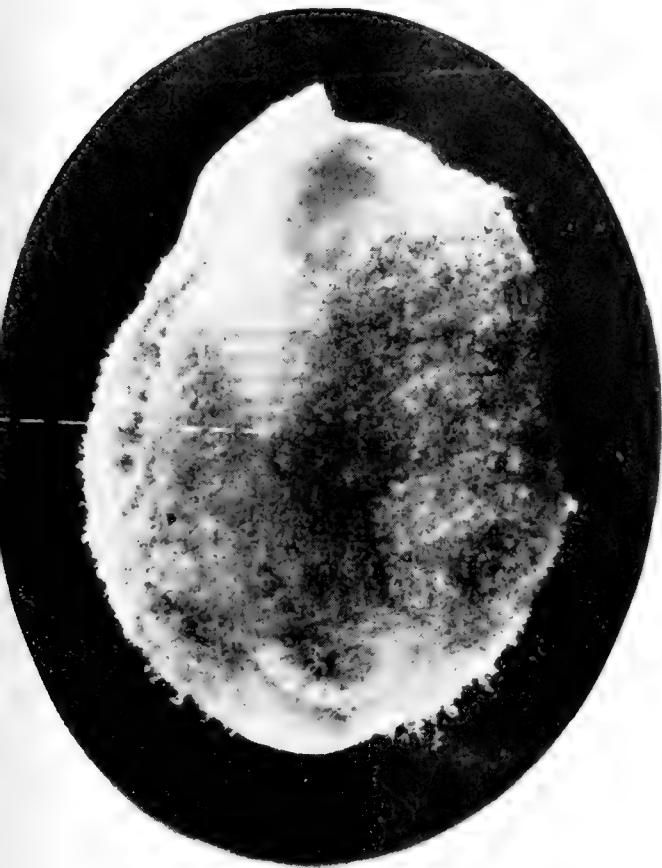
### EXPLANATION OF FIGURES

40. The dorsal view of a vesicle in a rather advanced stage of development. The embryos measure 155 mm. from tip to tip. The dorsal notch, *d.n.*, although extending down to near the middle of the vesicle, does not completely separate the lateral placental discs.  $\times \frac{2}{3}$

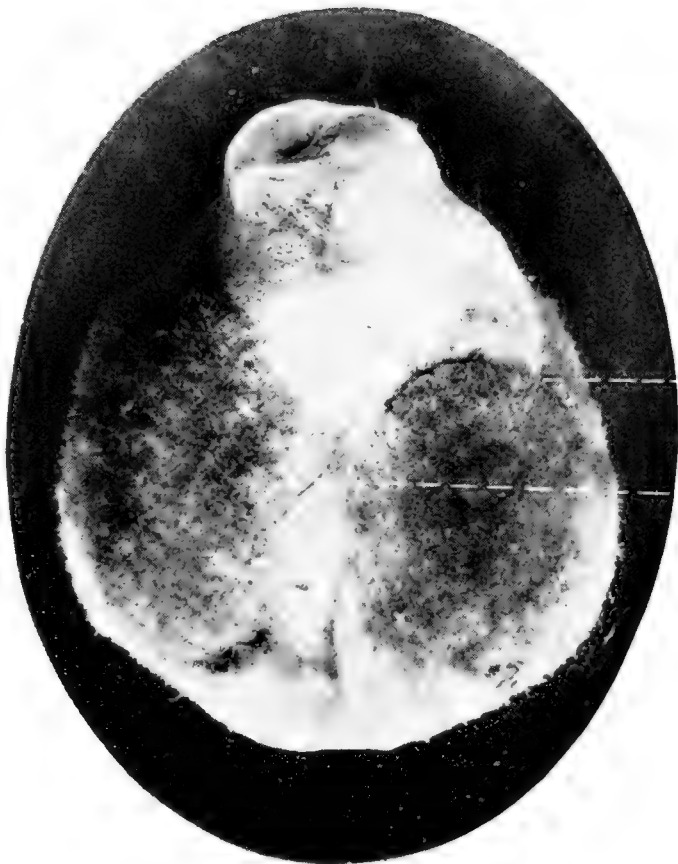
41. Dorsal view of a vesicle showing the definitive condition of the placenta. The placenta is divided into two lateral discs, each of which is distinctly bilobed. The notch between the two lobes of the left lateral (on right) disc is clearly shown in the photograph (*n.l.l.*). The discs are united to each other both on the dorsal and ventral side by placental bridges, the one on the dorsal side (*d.b.*) being the narrower. The original arborescent villi at the cervix end have greatly degenerated, and have become reduced to flat, blunt knobs. The embryos in this vesicle are about 210 mm. from tip to tip.  $\times \frac{1}{2}$ .

42. Right lateral view of a uterus showing a dorso-ventral bilobing. Embryos are 48 mm. long.  $\times \frac{4}{5}$ .

43. Ventral view of a pear-shaped uterus, which contained embryos measuring 52 mm. This and the preceding uterus show two of the several forms that have been observed.  $\times \frac{1}{2}$



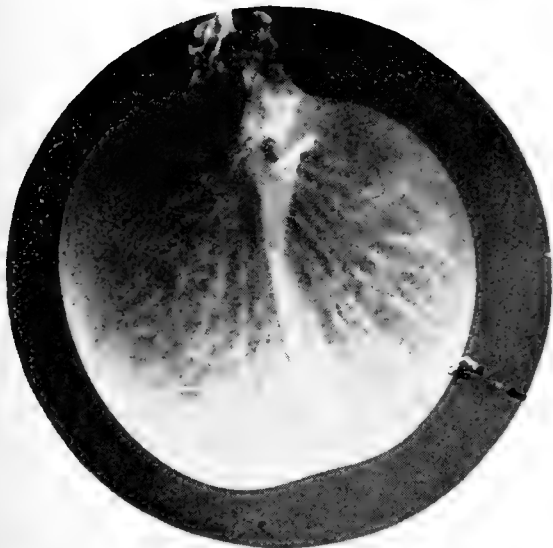
40



*n.l.l.*

*d.b.*

41



42



43

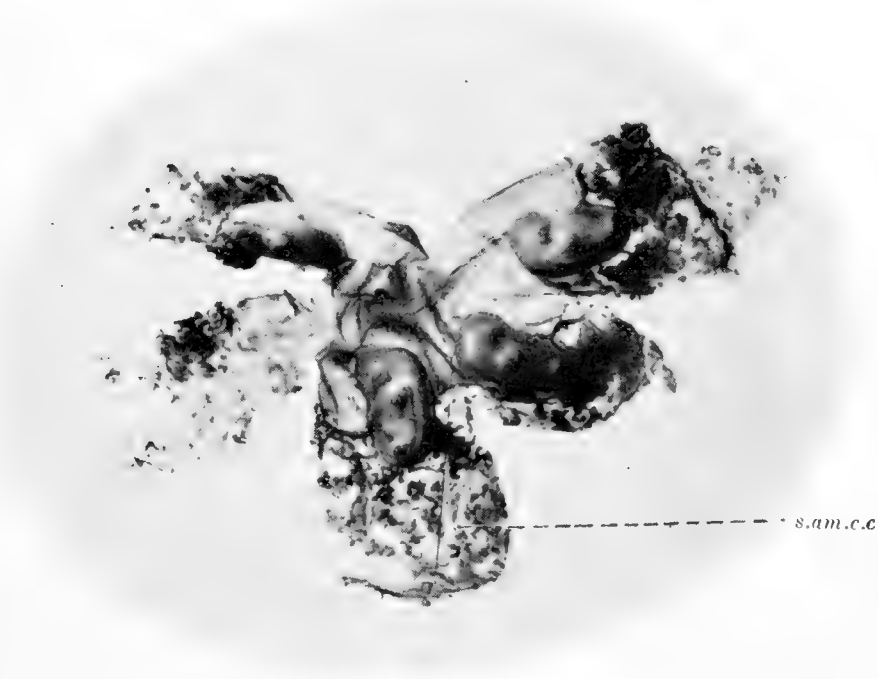


## PLATE V

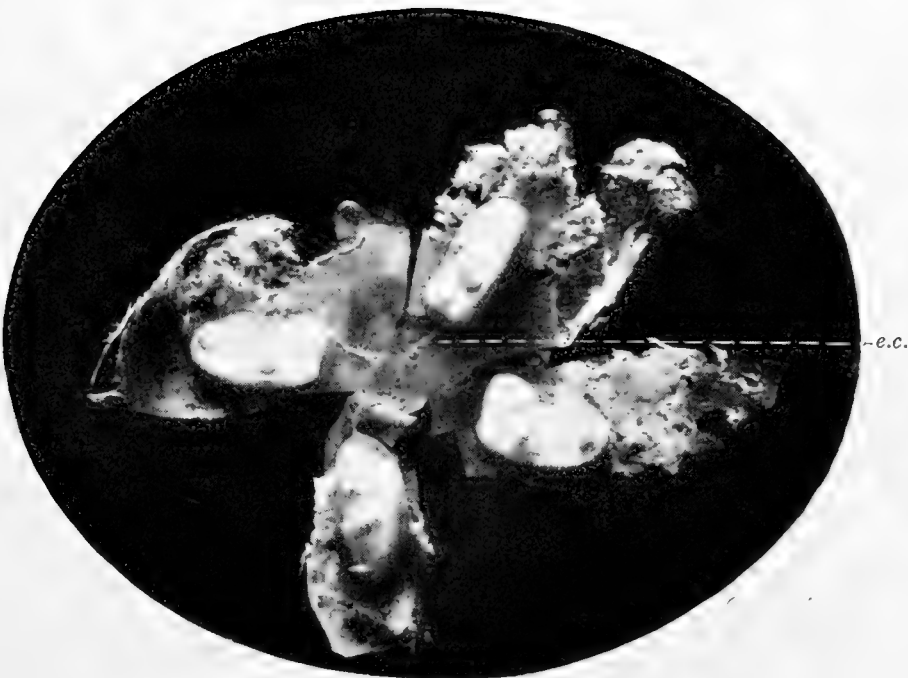
### EXPLANATION OF FIGURES

44. A vesicle split open to show the internal relationships of the different parts. The amniotic connecting canals are seen to pass from the anterior ends of the amnia to the spot occupied by the common amnion. This vesicle also shows a supernumerary canal (*s.am.c.c.*) extending from a small vesicle in the Träger wall to the canal belonging to the lower, right-hand embryo. In the entire condition the vesicle measured 24 mm. wide by 29 mm. long. (see fig. 3 for a diagram of the placenta.) Very slightly enlarged.

45. A vesicle laid open in a manner similar to the preceding. At the distal end of each canal is shown a series of bead-like enlargements (*c.e.*). The origin of the canal from the anterior tip of the amnion is shown with especial clearness in the embryo lying nearest the foot of the plate. In the entire condition the vesicle measured 24 mm. wide and 30 mm. long. Very slightly enlarged.



44



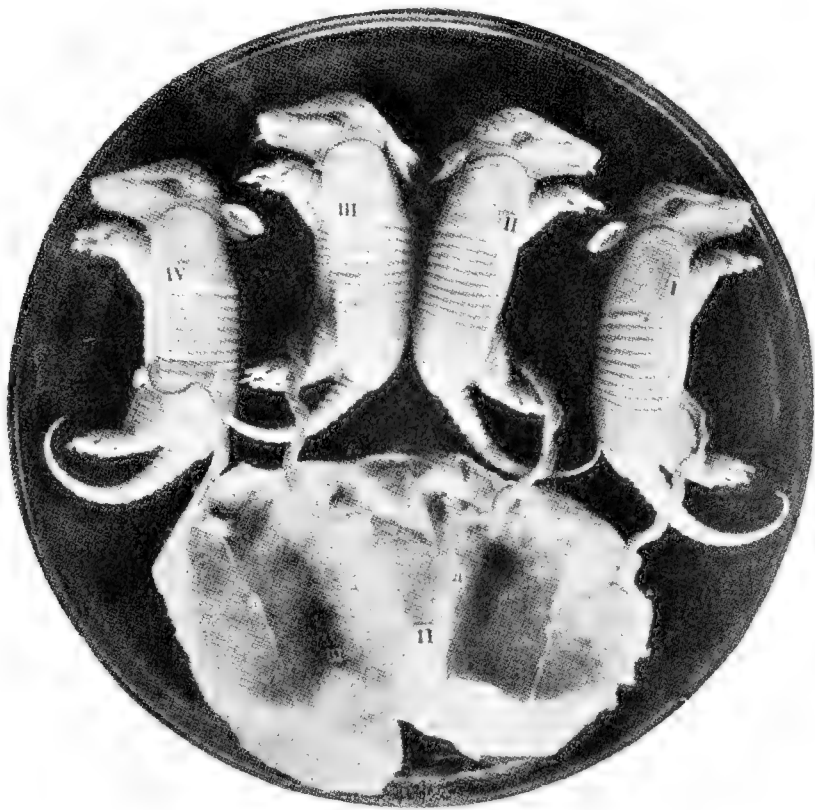
45

## PLATE VI

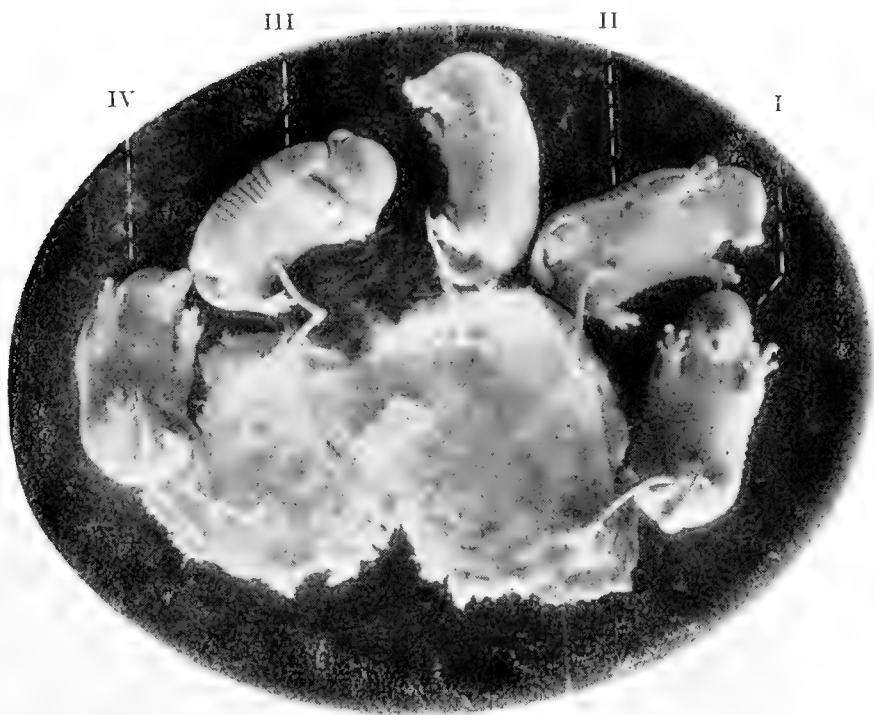
### EXPLANATION OF FIGURES

46. A vesicle cut open along the mid-ventral line to show the relationship of the embryos to each other and to the wall of the vesicle. Each of the four amniotic partitions (*a*), which have been cut off close to the chorionic wall, lies just to the left of the umbilical cord. These are attached to the wall near the tips of the placental lobes at the fundus end. The left lateral placental disc is indistinctly seen through the chorionic wall, and the notch separating it from the right lateral disc is marked with the larger "n", while that indicating its division into the two lobes is designated by the smaller "n,"  $\times \frac{1}{3}$ .

47. A photograph of vesicle no. 108, which contained five embryos. This vesicle was cut open along the mid-ventral line. Embryos nos. I, II, and II, are attached to the large, right lateral placental disc, and embryos III and IV to the smaller, left lateral. (See text for a fuller description and significance.)  $\times \frac{1}{2}$ .



46



47



# EARLY STAGES IN THE DEVELOPMENT OF THE CENTRAL NERVOUS SYSTEM OF AMBLYSTOMA PUNCTATUM

LELAND GRIGGS

*From Dartmouth College, Hanover, N. H.*

TWELVE TEXT FIGURES AND ONE PLATE

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## INTRODUCTION

The investigation of which this paper is an account was conducted in the Zoölogical Laboratory of Dartmouth College under the direction of Dr. William Patten. The method which Dr. Patten used in his studies of the development of the nervous system and sense organs of arthropods, an examination of external markings of specially prepared embryos of a very early stage, has been applied here to a study of these organs in *Amblystoma*. I wish to express my indebtedness to Dr. Patten for his suggestions upon my entering on the work and for his careful supervision throughout.

It is the purpose of this paper to offer a slight contribution to the solution of the problem of vertebrate cephalogenesis. The subject has been treated, however, in its narrower aspect strictly as a problem in amphibian embryology with such general references to wider questions as has been required to make clear the history of the work in this field of research.

## HISTORICAL REVIEW

Research in the field of amphibian embryology has already yielded results of considerable value. Neuromeres have been observed not only in the neural plate but also in the neural crests and the neural tube; the anlage of the lateral eyes has been traced back to pigment spots, and the early origin of the ear in relation to lateral line organs has been observed.

Before reviewing these topics, however, it will first be necessary to clear the way by considering two minor points, which have been the cause of considerable confusion, the closing of the blastopore and the formation of a series of grooves in front of the blastopore.

The closing of the blastopore has been made the subject of extensive research. It is agreed that the circular form by a more rapid inward lateral growth becomes an oval, then a narrow slit. The next change, however, is a matter of dispute.

Jordan ('93) finds that in the newt *Diemyctylus* the next step is a fusion of the walls of the slit for a very short distance either at the posterior end or at the anterior end or, as is more usual, at both ends at once. Next there is a fusion of the two walls at the center of the slit. Thus there are formed two temporary pores the distance between which is slightly less than the original diameter of the circular blastopore. The anterior of these two pores is the opening of the neurenteric canal and the posterior one becomes the anus.

Eycleshymer ('95) in his study of *Amblystoma* and *Rana palustris* confirms Jordan's statement of the origin of the anus and of the neurenteric canal but he does not describe any preliminary fusing at the ends of the slit-like blastopore. He finds that there is occasionally "a pear-shaped opening with the smaller end anterior instead of the usual dumb-bell outline." He describes the position of the anus when first formed as lying just outside the neural crest.

Morgan ('97) finds that in the *Urodeles* the anus is formed as described by Jordan and Eycleshymer but in the *Anura* here is a concrescence of the slit-like blastopore posteriorly and

the anus appears later as a new structure at the point where the posterior end of the blastopore disappeared, a condition which had already been described by Robinson and Assheton as "a reopening of a temporarily closed blastopore." Morgan was the first to observe that the thickened blastoporic lip was bounded by a definite groove, which he saw, however, only in front of the blastopore as a "sickle-shaped" depression. This thickened blastoporic lip is, according to Semon ('01), more prominent in *Ceratodus* where it forms a broad circular rim surrounding the slit-like blastopore, and this rim is bounded on the outer edge by a circular groove, a condition which, as will be shown, is very similar to that found in *Amblystoma*.

The closing of the blastopore leaves on the surface of the egg a narrow groove commonly called the primitive groove. This structure was confused by the earlier investigators with other grooves lying in front of it. Miss Johnson ('84) has recorded her observation of but one groove running over the surface of the egg for a distance equal to about three fourths of the length of the neural plate. She homologizes the groove with the primitive groove of higher vertebrates. Miss Johnson makes one statement which rather refutes her theory of the presence of but one groove when she says that "the front end of the primitive groove deepens into a distinct pit." It will be shown that in *Amblystoma* this pit is in reality a distinct groove which has an entirely different origin from the groove which arises from the closing of the blastopore.

Robinson and Assheton ('91) consider only the posterior portion of the long groove the true primitive groove, limiting the term to that portion of the groove which is formed by the lips of the blastopore. Jordan ('93) is more exact in his definitions. He holds that the first or primitive groove never quite equals the diameter of the original blastopore, and that it shows a fusion of the primary germ layers throughout its length, while the second groove shows no fusion of layers, although there is an "apparent fusion" at the anterior end where Miss Johnson found an anterior pit.



Eycleshymer ('95) in his observations on *Amblystoma* not only observed a distinction between neural and primitive grooves but he records a division of the neural groove into two parts. The anterior part of the neural groove usually appears first before the neural crests are clearly formed, and then "a second groove is often observed lying between the posterior end of the neural groove and the blastopore." This groove he calls the "dorsal groove." He notes that it is sometimes absent and again sometimes the neural groove appears to run forward "as a continuation of the slit-like blastopore." He considers the dorsal groove as really "a part of the neural groove having arisen in precisely the same manner." His figures indicate in later stages an anterior pit, but no mention is made of it. Eycleshymer has thus gone farther than any other writer in analyzing the single primitive groove of Miss Johnson into three grooves, "the primitive groove," "the dorsal groove," and "the neural groove."

More recent writers have failed to notice these three grooves. Morgan ('97) who adopts the old terminology applies the term "primitive groove" to the whole series of grooves in the frog, but he makes the significant statement that in older embryos "the primitive groove is narrower." It will be shown in this paper that this apparent narrowing of the old groove is in reality the appearance of a new groove after the disappearance of the old one.

Semon ('01) insists that in *Ceratodus* there is undoubtedly a lengthening of the groove which arises from the closing of the blastopore. It must be admitted that this is what would be expected since the groove lies in the growing region of the embryo. The condition in *Amblystoma* seems to resemble very closely the structure which he has figured for *Ceratodus*. Earlier authors who claimed that the primitive groove was shorter than the diameter of the original blastopore were correct so far as the first appearance of the groove was concerned, but Semon has shown that they overlooked the fact of a later growth which can be demonstrated in *Ceratodus* only by sections which show the typical fusion of the germ layers extend-

ing over a distance considerably greater than the diameter of the blastopore.

The nature of these grooves is not only in itself an interesting problem which is not yet wholly solved, but the relation of the grooves to the nervous system is also very important. It has quite generally been taken for granted that these grooves, one or all of them, are "neural" grooves, but this relation has never been well established.

In turning now to the more important problem of the early segmentation of the neural plate we find considerable divergence of views. Kupffer ('93) describes the cephalic plate of *Salamandra atra* as being divided into eight segments or primary neuromeres. The cephalic plate or "Hirnplatte" has no definite boundary posteriorly but appears to coincide in extent with the later "Archencephalon," which the author finds to be a rather vaguely defined vesicle comprising the region of the fore-brain, mid-brain and a part of the hind-brain. Froriep ('91 and '93) has made similar observations, although less complete on *Salamandra maculosa* and *Triton*. In the cephalic plate of the former he saw three or four segments, and in that of the latter he saw five, but in both cases there is an unsegmented tip which he thinks is large enough to represent three or four segments. Froriep, however, thinks this appearance of segmentation is merely due to the structure of the underlying mesoderm.

Later authors have made observations similar to those of Kupffer and Froriep, and, like them, have failed to agree on an interpretation. Eycleshymer ('95) finds in the neural plate of *Necturus* and of *Amblystoma* several large segments which, he considers, are caused by the mesoderm. Locy ('95) describes a few faint lines of division in the open neural plate of *Amblystoma* and four or five prominent divisions in the anterior end of the plate of *Rana palustris*, but since these divisions do not agree in number with the divisions of the neural plate which he considers to be true neuromeres he denies their metameric value. Hill ('00) has found in the solid anlage of the trout brain ten segments extending from the anterior tip of the plate to the

region of the anlage of the ear. Two of the dividing grooves appear deeper than the rest and so furnish convenient landmarks which no previous investigator had been able to find. The anterior of the two grooves proves to lie between the fore and the mid-brain, while the posterior one lies just back of the cerebellum. Thus Hill is able to show that there are three of these segments in the fore-brain, and two in the mid-brain. The general features of this segmentation were confirmed by the study of the chick of one somite although here the larger grooves were absent.

A comparison of the results obtained by these various authors is difficult, especially as to the number of segments, since Hill is the only one to find definite persisting landmarks. But the numerous observations as to the presence of some kind of division in the open neural plate is sufficient to warrant further research.

Turning now to the segmentation of the neural crests as they first appear at the sides of the neural plate we find that in *Amblystoma* embryos such a segmentation has been observed by Eycleshymer ('95) and Locy ('95). The former considers the appearance of segmentation merely an artificial scalloping due to the reagents used, because he finds variation among various embryos. The latter, however, regards the segments as true neuromeres, but he did little work on *Amblystoma* and that merely to confirm his work on *Acanthias*.

In *Acanthias* Locy ('95) finds a series of segments in the neural ridges which he is able to trace through the closing of the neural tube and the formation of the brain vesicles. The fore-brain contains three segments, the mid-brain two and the hind-brain nine. Hill ('00) has confirmed these observations in his study of the chick embryo. He finds that the grooves which separate the segments extend entirely across the neural plate. This is the only observation yet made showing that the segmentation of neural crests agrees with that of the open neural plate. The accuracy of this observation has been called in question by Kupffer ('03).

The segmentation of the closed tube in the region of the medulla was observed by the early anatomists and embryologists (Balfour, '81). These segments were first carefully studied by Orr ('87) in his work on *Anolis*. He was the first to use the term neuromere, and his description of a typical neuromere has ever since served as a criterion of a neural segment in the closed neural tube. He says: "Each neuromere is separated from its neighbors by an external dorso-ventral constriction, and opposite this is an internal sharp dorso-ventral ridge—so that each neuromere (*i.e.*, one lateral half of each) appears as a small arc of a circle. The constrictions are exactly on each side of the brain. The elongated cells are placed radially on the inner curved surface of the neuromere. The nuclei are generally nearer the outer surface and approach the inner surface only toward the apex of the ridge. On the line between the apex of the internal ridge and the pit of the external depression, the cells of adjoining neuromeres are crowded together, though the cells of one neuromere do not extend into another neuromere." He found six neuromeres in the hind-brain. The first and fifth have no nerve connection; the second is connected with the fifth nerve; the third to the sixth nerve; the fourth to the seventh and eighth nerves; the sixth to the ninth nerve. He considers that the mid-brain is a single neuromere and that the thalamencephalon comprises two neuromeres. He does not regard the secondary fore-brain as being a true neuromere. McClure ('90) was the first to claim that these neuromeres of the closed tube extended in a regular series to the anterior tip of the brain. He found that *Amblystoma* had one less neuromere in the hind-brain than Orr and Hoffman had found in reptiles. He considered that the difference was due to a fusion of two neuromeres. He found in the forebrain evidences of two neuromeres and possibly a portion of a third. Waters ('92) confirmed McClure's account of the absence of one neuromere in the hind-brain of *Amblystoma*, but in the teleost brain he found the whole number, in the hind-brain six, and assigned to the mid-brain two, and to the fore-brain three. Kupffer ('03) from the results obtained by McClure and Waters concludes that *Amblystoma* presents

an exception to the usual number of neuromeres in the hind-brain.

Very extensive observations on the neuromeres of the closed tube have been made by Locy ('95) and Hill ('00). Their work has been of especial value owing to the fact that they have shown the direct correspondence between the segments of the neural tube and those of the neural crests before the tube is formed. By following carefully the history of the segments in the anterior end of the crests they observed in the anterior end of the neural tube a few transitory segments which had not been seen before. Locy found in the fore-brain of *Acanthias* three segments, in the mid-brain two and in the hind-brain nine, counting three in the vagus region which are not included in the brain by earlier writers. Hill in his work on the trout and chick confirmed Locy's investigations. Johnston ('05) also claims to have confirmed these results in his work on *Amblystoma* which he has not yet published.

Thus we have presented to us by Locy, Hill and Johnston a fairly complete account of the neuromeres of the brain. According to this account there are eleven similar divisions in the anterior end of the neural plate which constitute a cephalic plate, not, however, marked off from the rest of the plate except by their subsequent history. The segments extend laterally across the neural crests and after the tube is formed the divisions extend completely around it. The segments in the hind-brain are those already investigated by earlier authors. They persist until the nerve relations can be fairly well determined. In the fore-brain and mid-brain, however, the segments are transitory. In the fore-brain they completely disappear before the differentiation of the secondary fore-brain and thalamencephalon.

Kupffer ('03, '05) has recently described a series of segments, his so-called secondary neuromeres, which he has shown to be quite generally present in vertebrate embryos. His fore-brain divisions telencephalon, parencephalon, synencephalon, although they agree in number with Locy's divisions, are evidently not the same, for Locy has shown that his divisions disappear before there is any trace of this later differentiation of the fore-brain.

Kupffer's first and second mesencephalic neuromeres, however, are evidently the same as Locy's. Kupffer, as has already been stated, considers these divisions described by Locy and himself as of secondary origin and not true primary neuromeres, maintaining that the true neuromeres are the large divisions in the open neural plate which he has described as being apparent in some forms of amphibians.

Neal ('98) is the severest critic of the theory of neuromeres as developed by Hill and Locy. After an investigation of *Acanthias*, the same form which Locy used, he disagreed with Locy both as to observation and interpretation. He found that "the lobes on the opposite sides of the plate do not correspond in number or position, neither do they show any definite relations to the mesodermal somites," and "there is no constancy in different individuals." He found these apparent segments transitory and was unable to find any relation between them and the later ventral segmentation of the neural tube. He pointed out another apparent inaccuracy in Locy's work in that "the line which separates the expanded cephalic plate from the region posterior to it marks the posterior boundary of the auditory invagination," instead of lying "just in front of the point where subsequently the vagus nerve begins" as Locy saw it. From this he concludes that Locy can not have traced his so-called neuromeres correctly into the later divisions of the brain.

Neal accepts Orr's criteria of a neuromere but he adds the following important point—"the best criteria are such as associate the supposed neuromeres metamerically with other structures known to be segmental." Judged by this standard he concludes that the fore-brain and the mid-brain represent each one neuromere, the former being associated with the anterior head cavity of Platt and the sensory olfactory nerve, and the latter with Van Wijhe's first somite and the ophthalmicus profundus of the fifth nerve. Neal's method is undoubtedly very valuable in showing positive evidence in favor of the presence of true neuromeres in the hind-brain, but it is very difficult to apply so severe a test to the mid-brain and the fore-brain because it has been demonstrated so clearly that the nerve com-

ponents and the mesodermal somites of this region have undergone profound alterations. If, as the most recent investigation seems to show, the nervous system, appearing first, presents a simpler and more unaltered condition than the other two systems, then it may well serve as a basis for the study of segmentation of the head; and other organs should be shown to correspond to it rather than *vice versa*.

From this survey of the literature dealing with the segmentation of the nervous system of the Amphibia and allied forms it seems fair to conclude that the presence of neuromeres has been proved conclusively. As Hill ('00) says, "There is substantial agreement among observers as to the six segments in the hind-brain." The problem of the segmentation of the fore-brain and mid-brain, however, may still safely be considered unsolved. While the evidence in favor of the segmentation of the neural crests and the open plate seems very strong, the difference of opinion as to the constancy, number and relations of the segments is so great that considerable more research will be necessary before we have a fully established theory of primary neuromeres.

In solving the problem of the morphology of the vertebrate head the subject of sense organs is second in importance to that of neuromeres. The origin of the olfactory organ, of the eyes both lateral and parietal, of the ear and the lateral line organs have all been made the subject of careful investigation, particularly in the Amphibia and other groups among the lower vertebrates. For the purposes of this paper only one of these topics will be considered in detail, that of the origin of the lateral eyes.

It was observed by Balfour ('81) that the anlage of the eye appeared before the closing of the neural canal. He has quoted with approval a statement by Lankester that the "original vertebrate must have been a transparent animal with an eye or pair of eyes within the brain."

Whitman ('89) observed the anlage of the eyes on the surface of the amphibian egg in the open neural plate stage. In *Necturus* before the neural crests have begun to move together he found that "the basis for the eye is already discernible as a cir-



cular area." Whitman has proposed a theory which to-day attracts more attention than Lankester's theory, although it will be seen that the two theories do not necessarily conflict. His hypothesis is as follows: "The medullary plate of the vertebrate is undoubtedly an enormous extension of the ancestral invertebrate plate. Sense organs lying originally outside the neural plate have probably in consequence of this extension in width, been brought within the medullary area."

Eycleshymer ('94) continued Whitman's work on the origin of the eyes of the Amphibia. Of the three forms which he studied, *Necturus*, *Amblystoma*, *Rana palustris*, he found the last named the most satisfactory for his purpose on account of the deeper pigment of the eye spots and their greater histological differentiation. He studied very fully the histology of the pigmented areas, but he leaves it a little in doubt from his plates and descriptions whether those areas are actually on the neural plate or between the plate and the crests, a very important point in testing Whitman's theory that the ancestral invertebrate plate has widened out so far as to include the region of the lateral eye. His conclusion in favor of the hypothesis that the vertebrate eye must have been located originally within the brain, and his statement in support of this theory that "this was precisely the case in *Rana palustris*," must be taken to mean that the original brain comprised both neural plate and neural crests.

In criticism of this theory of the relation of the eyes to the primitive brain it may be suggested that the neural plate proper represents the central nervous system of the ancestors of the vertebrates, and that the anlage of the vertebrate lateral eyes are located between the neural plate and the neural crests. If this be true it is probable that the medullary plate has not undergone such an enormous extension as Whitman supposed and that the ancestral vertebrate eyes were not located on or in the brain, as Lankester and Eycleshymer supposed, but on the margin of the neural axis, as in arthropods, and that by a folding over of the non-nervous sides and roof of the brain they became included in the neural canal in a similar way to that described by Patten for the arachnids (Patten, '89). Such a view as this obviates the necessity of assuming that the nervous tissue in-



creased in bulk by appropriating other tissues. To prove the correctness of this view it would be necessary to show that the anlage of the eyes does not lie on the neural plate. If we turn to work outside the field of amphibian embryology for evidence we find that the neural plate is not very sharply marked off from the neural crests in those animals which have been studied most. Fororiep ('05) locates the "Sehgrube" of *Torpedo* on the edge of the neural plate in the area called by His "Flugelplatte," and Locy ('95, '97) locates the optic groove of *Acanthias* and the chick on the sides of the neural furrow as the walls begin to rise to form the canal. None of these forms, however, has a clearly marked groove between the neural plate and the neural crest and therefore, although these authors seem to believe that the eye spot is located on the anlage of the brain, it is clear that this interesting point still remains unproved.

Locy's work on *Acanthias* is especially interesting inasmuch as he was the first to find "accessory optic vesicles." He observed that the first, or true optic groove, covered the space of three neuromeres and following this was a series of five or six accessory vesicles occupying the space of one neuromere each, although this relation to neuromeres was not always strictly observed. Locy has compared this arrangement with that of the same organs of the leech embryo described by Whitman ('89). In the process of development the accessory vesicles degenerate except the first pair which according to Locy form the pineal eye. A study of the chick confirmed the work on *Acanthias*.

This review of the literature dealing with the problem of the development of the nervous system of the Amphibia has revealed the need of further research along several lines. First, the series of early grooves which appear at about the same time as the neural plate should be carefully investigated and their relation to the nervous system determined. Then attempts should be made to trace Kupffer's primary neuromeres into the later divisions of the brain, and the exact position of the anlage of the eye should be determined and also its relation to numbered neuromeres.

## DEFINITION OF TERMS

Since, as has been shown in the preceding review, authors have not agreed upon terms and since many of the old terms have proved to be misleading it seems necessary to adopt a definite system of nomenclature. The terms used in this paper are as far as possible those of earlier writers. Where a choice between two old terms was made, or where a new term was chosen, it was done with the purpose of showing the position and relations of the structure involved without suggesting doubtful homologies.

In the first place the common term, primitive groove, has been avoided altogether. This word has been used in various senses by different authors, and furthermore it suggests a doubtful homology which it is not the purpose of this paper to discuss. The groove formed by the closing blastopore will be called the blastogroove. The two grooves lying in front of the blastogroove which have frequently been called the primitive groove will be called the anterior and posterior germinal depressions. Since these terms are perfectly colorless as regards homology they leave the way clear for an unprejudiced discussion of the meaning of the structures.

The term, neural groove, which has often been applied to one or all of these grooves just mentioned is not used in this connection, because the neural nature of these three early grooves has not been demonstrated. The true neural groove is a later structure appearing after the other grooves have begun to degenerate and persisting as the faint narrow groove lying in the bottom of the neural canal.

The term, neural plate, will be used in its usual sense. The division of this plate into well defined regions demands further definition. The terms, hirn-platte and cephalic plate, which have been loosely applied to the widened anterior end of the plate, will be discarded for the term procephalic lobes, denoting that part of the neural plate which lies in front of a distinct transverse furrow which will be called the transverse cephalic groove. The region behind this groove may be theoretically

divided into metencephalic plate and spinal cord plate, although in *Amblystoma* there seems to be no trace of a dividing groove.

Connected with the neural plate are some less important structures which should be defined. The narrow groove bounding the neural plate will be called the peripheral groove. The low ridge running around the neural plate just outside the peripheral groove will be called by its usual name of neural crest.

In *Amblystoma* the neural plate undergoes at an early period a sharp downfolding. This will be called the infundibular depression.

The term neuromere is one that needs careful definition. In this paper it will be applied to the early divisions of the neural plate which have been called primary neuromeres. These divisions are considered as having true metameric value. The later divisions of the neural tube will be called neuromeres in so far as they can be shown to be identical with the divisions of the open neural plate. The use of such terms as primary and secondary neuromeres will be avoided because they are contradictions of the idea that a neuromere is a genuinely segmental structure.

To sum up, the old terms neural plate, neural crest and neuromere will be used substantially as they have been used before, while the term neural groove will be used in a new and restricted sense. The new terms, blastogroove, anterior germinal depression, posterior germinal depression, peripheral groove, transverse cephalic groove, procephalic lobes, infundibular depression are new terms applied to newly discovered structures or to newly discovered divisions in old structures.

#### MATERIAL AND METHODS

*Amblystoma punctatum* was the animal chosen for study, mainly on account of the large egg which when first laid is about two mm. in diameter. This is larger than any frog's egg and much larger than the egg of *Amblystoma tigrinum* but considerably smaller than the egg of *Necturus*. The anlage of the nervous system of *Amblystoma punctatum*, however, is

nearly as large as that of *Necturus* and the markings on the neural plate are much more distinct.

The abundance of eggs also makes *Amblystoma punctatum* a good object for study, for, as Locy has already pointed out, in the study of neuromeres a very large supply of material is essential. In the vicinity of Hanover, N. H., the eggs are very common in the early part of April, being found in large bunches in all the small ponds and pools and even in the shallow ditches by the roadside. In the years 1903, 1904 and 1905, between three and four thousand eggs were collected for this study.

In fixing the embryos several fluids were used, chromic acid, picrosulphuric acid, Perenyi's fluid. All three of these fluids proved to be useful for various purposes. For the study of surface views chromic acid gave the best results. The eggs after being dissected out of the main mass of jelly were dropped into a 1 per cent solution. After remaining in this fluid for about half an hour they were freed from the outer envelope by the use of fine pointed forceps and needles and then transferred to a fresh solution of the same strength where they were allowed to remain for about four hours. The material was then thoroughly washed in running water for several hours and finally transferred to 70 per cent alcohol. The alcohol was changed as often as it became turbid. Before the eggs were ready for study they were immersed for a few minutes in a weak solution of eau-de-Javelle, which removed the inner membrane and the last traces of any albuminous precipitate. This treatment gave beautiful surface preparations. For dissection Perenyi's fluid was better. After older embryos had been fixed in this fluid for several hours and then hardened in 70 per cent alcohol it was possible to dissect out the brain with fine needles and sweep it clean with a delicate brush or the brain could be dissected with the sense organs and the ganglia left attached to it. For paraffin sections Kleinenberg's picro-sulphuric acid was the most satisfactory for fixing embryos.

The division of the embryos into a series of stages proved to be a difficult task. A single set of eggs killed at one time contains several stages, making it necessary to examine the eggs

one at a time. The embryos were first divided roughly into the conventional stages such as yolk plug stage, open neural plate stage. These stages, however, owing to the fact that the markings of the open neural plate are very transitory, had to be subdivided.

Some difficulty was encountered in thus subdividing the principal stages owing to a remarkable variation in the younger embryos. Some of the grooves and infoldings were so deep that abnormalities seemed at first sight to be very common. Further study of these variations showed that they were not promiscuous abnormalities but variations along certain well defined lines. Those structures which in a majority of embryos were faintly marked, and in a few embryos were not seen at all, were very plainly marked in others. The deeply furrowed specimens were, therefore, the most valuable for study when it could be shown, as it usually could without difficulty, that the deep furrows correspond in extent and position with the faint furrows in other eggs. Patten has already pointed out in his study of *Limulus* embryos the principle that in the process of recapitulation those embryos which vary from the normal average type may show best of all some features of the ancestral condition. This seems to be especially true in a study of neuromeres. Some embryos seem to show no neural segmentation in the early stages, the majority show it faintly, a few show it very distinctly, but in all cases the number of neuromeres is the same where they show at all and the size of the neuromeres is approximately the same. Hence we are obviously not dealing with a meaningless variation, but we are justified in making a careful study of the most clearly sculptured neural plates.

A second difficulty in the way of dividing the embryos into a series of stages is found in the fact that several distinct structures are forming on the surface of the egg at the same time and with a relative rapidity which varies with the different eggs. For example, if the eggs are assorted according to the closing of the blastopore the stages will not show a consecutive development of the neural plate, for among the eggs with a wide open

circular blastopore there may be some that show the outline of the neural plate and others that show no trace of the plate. This difficulty becomes much greater when the several distinct parts of the neural plate are taken into consideration.

The following division into stages is based upon the development of the particular structure which is made the special object of study in the general period under consideration. Thus the eggs before the appearance of the neural plate are divided into three stages; these stages depending upon the relative development of the germinal depressions, not upon the condition of the blastopore. After the appearance of the neural plate, however, the peripheral groove, the transverse cephalic groove, the neural crest and the neuromeres are successively the landmarks for division and it is necessary to ignore the germinal grooves, for they show many degrees of degeneration in each stage. After the closing of the neural canal these peculiar conditions are no longer found and assortment into stages becomes comparatively simple.

The method described above will be made clear by the following summary of stages.

#### DIVISION INTO STAGES

Stage 1. The posterior germinal depression has appeared in front of the blastopore. The blastoporic rim is bounded by a faint narrow groove.

Stage 2. The anterior germinal depression has appeared and the posterior depression shows signs of degeneration from behind forward.

Stage 3. The two germinal depressions appear very closely united. In a few eggs the blastogroove is now formed.

Stage 4. The area of the neural plate has become marked off by the peripheral groove. The condition of the germinal grooves and of the blastogroove varies.

Stage 5. The neural crests have appeared and there are indications of the transverse cephalic groove.

Stage 6. The procephalic lobes become divided into neuromeres.

Stage 7. The first neuromere folds down to form the infundibular depression.

Stage 8. The neural crests move together and fuse to form the neural tube.

Stage 9. The neural crests become segmented. The otic pit appears.

#### DESCRIPTION OF EMBRYOS

Stage 1 (Fig. 1, B). The posterior germinal depression (pgd fig. 1, B), the first structure to appear on the surface of the egg in front of the blastopore, is formed while the egg is still spherical before the yolk plug has been withdrawn. There are no indications of any growth in length of the depression, or of any origin at a constant point from which it grows either anteriorly or posteriorly, but the groove appears at the very outset as a long shallow depression of uniform length in different eggs.

In order to distinguish between this groove and the grooves which appear later it is important to note carefully its character. It extends from a point an appreciable distance in front of the blastopore to a point about half the distance across the upper hemisphere of the egg. It is nearly as wide as the diameter of the blastopore just before the latter changes from its circular to its oval form. Its depth varies owing to the presence of several shallow pits one of which is usually found at the anterior end forming a fairly well marked anterior limit to the depression.

It has already been mentioned that there is considerable difference of opinion in regard to the number of grooves which appear on the surface of the amphibian egg. Miss Johnson ('84), Schultze ('88) and Erlanger ('90) have maintained that there is but one groove, the primitive groove, formed by a concrescence of the lips of the blastopore and then extending forward over the surface of the embryo. A study of these early stages of *Amblystoma* supports the position taken by the later authors, Robinson and Assheton ('91), Jordan ('93), and Eycleshymer ('95), that there are two grooves, the posterior one derived from the blastopore showing a fusion of cell layers and the anterior

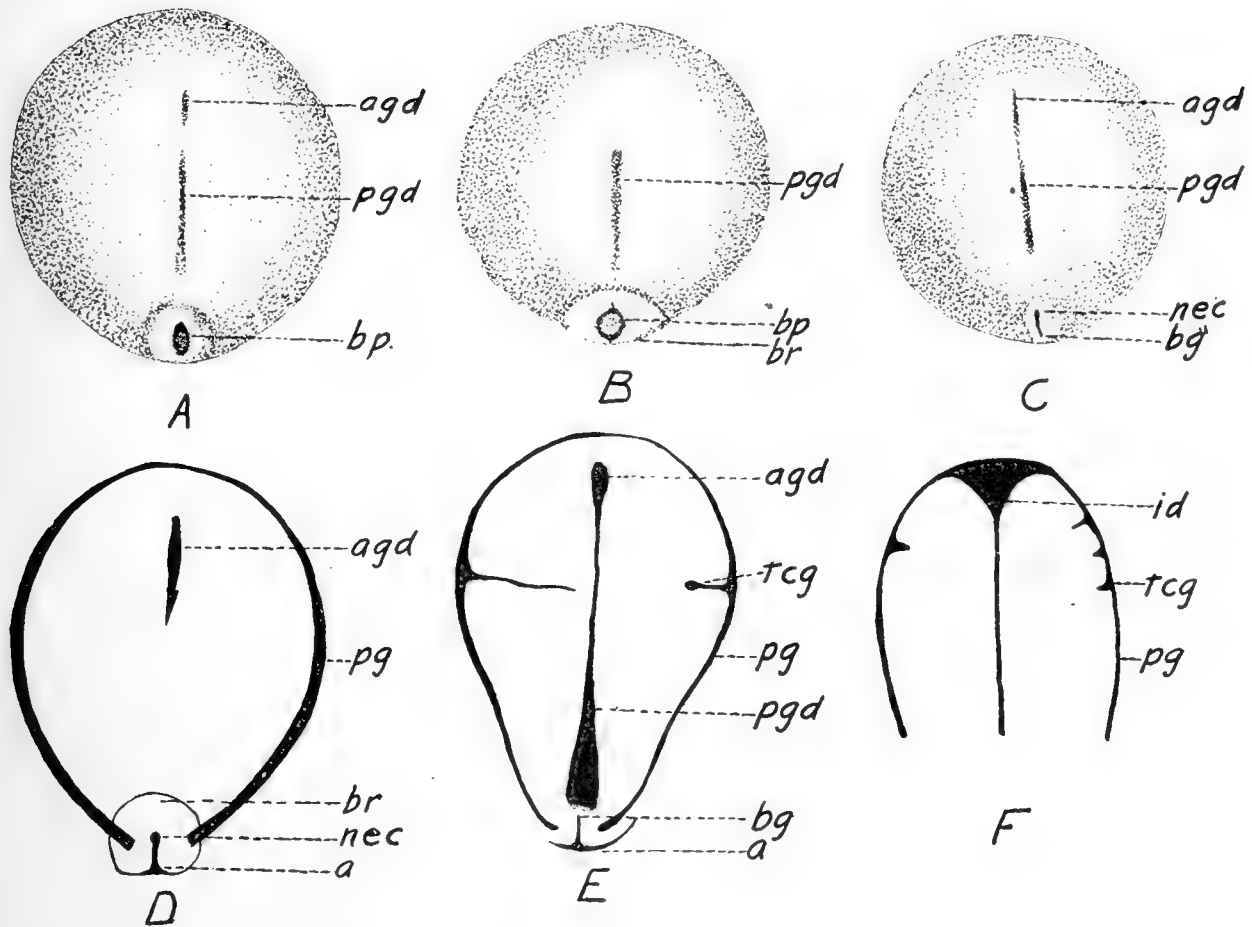


FIG. 1. Surface views of the first four stages. A, B, C are views of entire embryos and D, E, F are outline drawings of neural plates. These and the remaining figures of this paper were drawn with the aid of the camera. a, anus; agd, anterior germinal depression; bg, blastogroove; bp, blastopore; br, blastoporic rim; id, infundibular depression; nec, neurenteric canal; pg, peripheral groove; pgd, posterior germinal depression; tcg, transverse cephalic groove.

one showing no fusion. In *Amblystoma* the evidence for this view is still stronger; for when the posterior germinal depression is formed the blastopore is still wide open, and furthermore there is an appreciable distance between the posterior end of the depression and the anterior rim of the blastopore. It is not the purpose of this paper to enter into a further discussion of the morphology of the so-called the primitive groove. It is desired merely to emphasize the fact that the groove which is here called the posterior germinal depression arises independently of the blastopore.



Stage 2 (Fig. 1, A; Plate I, fig. 1). The posterior germinal depression now shows some changes (pgd fig. 1, A). It is narrower, deeper and more clearly defined since the shallow pits have disappeared. The anterior end has not changed in position, but the posterior end has begun to widen out and disappear leaving a considerable distance between the closing blastopore and the posterior germinal depression.

A new groove, the anterior germinal depression (agd) a structure very constant in its nature and present in all eggs of the right stage, has now appeared in front of the posterior depression. In the photograph (Plate I, fig. 1) the depression is not so sharply defined as in the figure. It becomes more and more sharply defined in succeeding stages, however, as may be seen by examining the photographs of older embryos. It is evidently not one of the pits described in the preceding stage since it is deeper, narrower, and longer, and furthermore the pits of the posterior depression have all disappeared at this time. These facts together with its very important later history give sufficient ground for considering it a separate and distinct structure.

This anterior germinal depression is evidently the groove which Eycleshymer ('95) in describing the early development of *Rana* and *Amblystoma* has called the neural groove proper, which he found to be connected with the groove derived from the blastopore by a third groove which he called the dorsal groove, evidently identical with the posterior depression described above. Morgan ('97) for *Rana* has figured the posterior part of what he has named the primitive groove as forming first, and later the anterior part appearing as a continuation of the part first formed. Evidently he observed the same process as Eycleshymer has described more fully. Neither of these authors, however, has described in detail the character of these two grooves nor traced their later history.

The value of adopting the new terms anterior and posterior germinal depression must now be apparent. It has just been shown to be a mistake to call the posterior depression a primitive groove, if by that term is meant a groove derived from the blastopore. It is obviously just as much a mistake to call the

anterior depression a neural groove since it forms before any well defined anlage of the nervous system appears and when its later history, as will be shown, shows no particular relation to the nervous system. The term germinal depression does not, by begging the question at the very outset, confuse the problem of the morphology of the various grooves, but leaves the field clear in which to follow the history of these important structures.

Stage 3 (Fig. 1, C. Plate I, fig. 2)). This stage shows the three early grooves present at one time. At the anterior end lies the anterior germinal depression, in the middle the posterior germinal depression, and at the posterior end of the series the blastogroove. These three grooves are distinguished from one another not only by order of appearance and position but also by nature of the grooves themselves as will be shown later by means of transverse sections.

The anterior germinal depression (agd, fig. 1, C) shows some changes when compared with the preceding stages. It is narrower, deeper and longer and is more closely united with the posterior depression. In fact in this stage it is more difficult than at any other time to distinguish between the two depressions. Yet this is possible in every embryo, the majority showing the distinguishing features much better than the one illustrated in Plate I, fig. 2. By reference to fig. 1, B and E, typical representatives of their respective stages, it is seen that although it may be difficult to locate the exact point of union of the two grooves, yet the grooves are readily distinguishable from each other by a difference in width and depth.

The posterior germinal depression shows further signs of degeneration by widening out and disappearing at the posterior end. Scattered shallow pits again appear in an irregular manner as in the first stage

The blastogroove is formed by the concrescence of the lateral lips of the blastopore. This groove together with the neurenteric canal can be described better in connection with one of the older stages. It may be noticed here that the neurenteric canal (nec) forms the anterior limit of the blastogroove and that the

latter's length is not greater than the diameter of the circular blastopore.

Stage 4 (Fig. 1, D, E, F. Plate I, figs. 3, 4, 5). From this time on the three grooves, the development of which has just been traced, undergo rapid modification. The posterior germinal groove shows unmistakable signs of degeneration in all eggs of this stage. In fig. 1, D, and in Plate I, fig. 3 there is no sign whatever of the original groove, a condition which is found in about one-third of the eggs. In a few eggs, although the groove has disappeared, there is a faint dark line marking its former position.

The only remaining portion of the anterior germinal depression (agd) in fig. 1, D, is a short deep groove or rather pit, and this deeper portion of the original groove may be seen in some eggs before the disappearance of the main part of the groove (agd, fig. 1, E). Miss Johnson ('84) seems to have been the first one to describe this "anterior pit" as she calls it but she failed to notice the series of germinal grooves, apparently considering the anterior pit as a part of one long primitive groove. Eycleshymer's ('85) figures of *Necturus* show a similar structure and he seems to have considered the pit a part of the "neural groove." His figures as well as those of Miss Johnson are representations of older embryos than those of this stage of *Amblystoma* now under consideration, and the single long groove evidently corresponds to the neural groove proper which will be described shortly. Hence their figures showing the relation of the pit to a single long groove are correct. The history of this pit, however, as will appear from an examination of the various stages shown in fig. 1, indicates that it is in reality the deeper and persisting part of the anterior germinal depression. A study of succeeding stages corroborates this view.

The neural plate in this stage is marked off by a peripheral groove (pg). This new groove does not take its origin from a given point from which it grows forward or backward but from the first it extends for a considerable distance along the sides of the plate and, although very faintly, across the anterior end of the plate. It cannot at its first appearance be traced around the posterior end of the plate. As the photograph and the

figures illustrating this stage show, the changes which develop in this groove consist of a widening and deepening and a growth backward toward the blastopore.

Lying in the peripheral groove at a point opposite the posterior end of the anterior depression is a pit or short groove (tcg, fig. 1, E, F), one on each side of the neural plate. This pit is the beginning of the broad transverse cephalic groove which later marks off the anterior end of the neural plate from the posterior end. It is visible in a majority of the eggs as soon as the peripheral groove appears but in a few instances as in fig. 1, D, no trace of it appears in this stage. Not only is the anterior end of the plate, the procephalic lobes as this region may be called, marked off by the groove but also, when the eggs are hardened in chromic acid, by a striking difference in color. The procephalic lobes are darker than the remainder of the plate, a distinction which remains until about the time of the closing of the neural tube. Thus at a very early stage color and a definite boundary mark out the region which proves to be the anlage of the brain. The transformation of this anlage, the procephalic lobes, will be the principal object of attention in the succeeding stages.

Stage 5 (Figs. 2, 3, Plate I, figs. 6, 7). This stage is characterized by the appearance of the neural crest (nc, fig. 2). The crest first appears as a pair of short longitudinal thickenings at the sides of the neural plate opposite the transverse cephalic groove (fig. 2, E). Each half of the crest grows rapidly backward until it reaches the blastoporic rim (br, fig. 2, A) which in a few eggs is still distinctly visible. After the disappearance of the blastoporic rim the two sections of the crest fuse behind the neural plate (fig. 2, B). At a slightly later period the crest is continued around the anterior end of the plate (fig. 2, C). This description of the origin of the neural crest agrees with the more general statement of Eycleshymer ('95) that in the Amphibia the neural "bands arise independently" and "differentiate in situ."

It has been quite generally assumed that the neural crest which surrounds the open neural plate is identical with the later

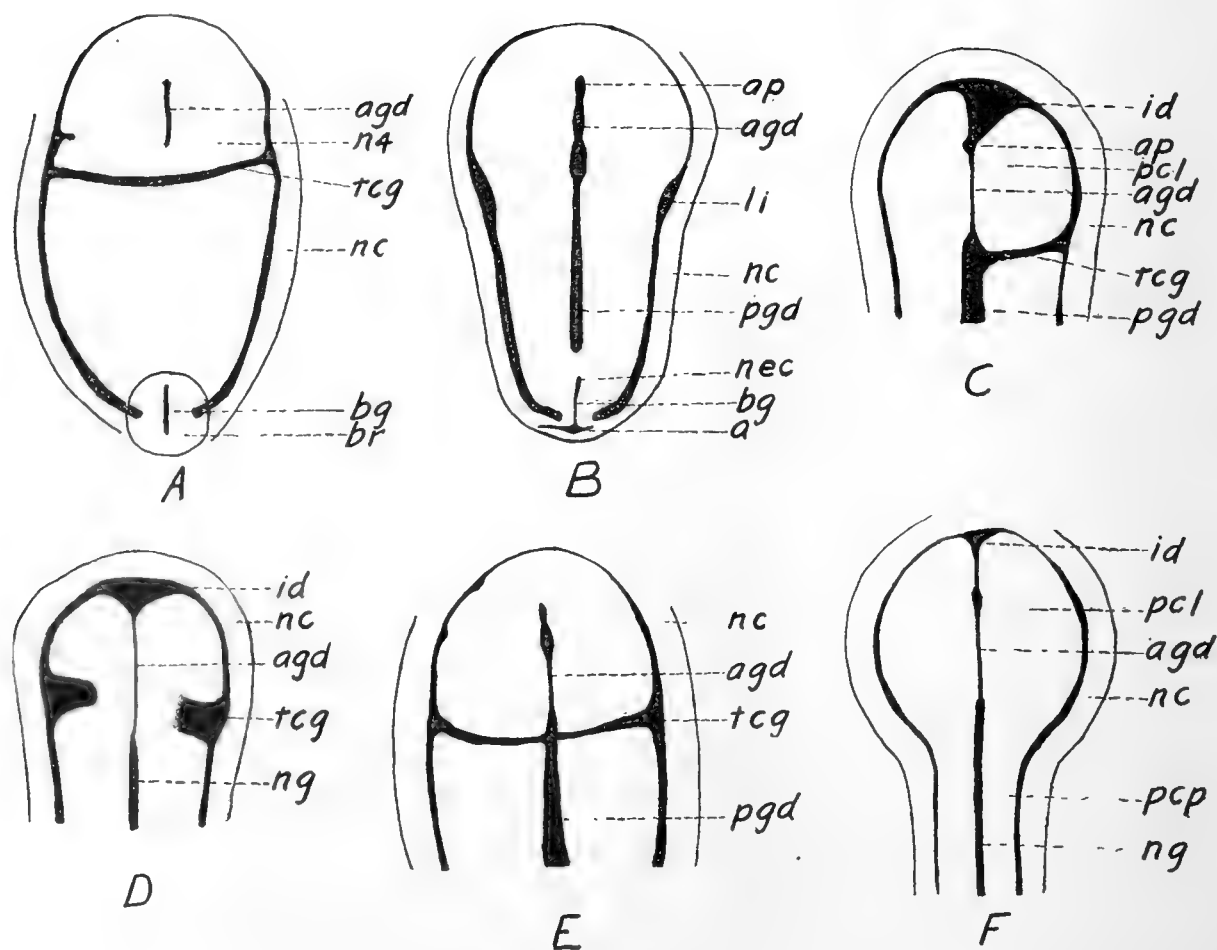


FIG. 2. Neural plate and neural crest just before the appearance of neuromeres, fifth stage. a, anus; agd, anterior germinal depression; ap, anterior pit; bg, blastogroove; br, blastoporic rim; id, infundibular depression; li, lateral infolding; n4, fourth neuromere; nc, neural crest; nec, neurenteric canal; ng, neural groove; pcl, procephalic lobes; pcp, postcephalic plate; pgd, posterior germinal depression; tcg, transverse cephalic groove.

neural crest from which the dorsal ganglia of the spinal nerves arise. Almost conclusive evidence is found in the fact that the crests in the open plate and in the closed tube hold the same position, lying in both cases immediately adjacent to the edges of the plate or to their line of fusion in the tube. Johnston ('05) has recently shown by a series of sections that the rounded or cubical cells of the crest in the open plate stage can be easily followed into the anlage of the dorsal roots of the cerebral nerves. His sections are drawn from *Amblystoma* embryos. My obser-

vations agree entirely with his. It will be assumed then in any further consideration of this subject that the generally accepted position in regard to the identity of the neural crests at the various stages is correct.

The relation of the neural crests to the neural plate as pictured in fig. 2 at once suggests the hypothesis of the longitudinal zones of the brain which has been elaborated in detail by Johnston ('02, '05), but this particular theory of the neural crest is not supported by the conditions just described. From the fact that in *Amphioxus* the greater part of the nerve elements homologous with the neural crest lie inside the neural tube and that many of these elements are found there in fishes and even in *Amphibia*, Johnston draws the inference that the neural crest was originally a part of the central nervous system and that there "has been a progressive separation of material for the ganglia from the brain." It has just been shown, however, that the neural crest develops at a later period than the neural plate and that the two structures are separated by an appreciable distance. Furthermore it will presently be shown that the crest and plate are not segmented in the same way, a fact already noted by Locy ('95). The evidence from the embryology of *Amblystoma* then does not show a close relation between neural plate and neural crest but rather it seems to indicate that the plate represents the anlage of the central nervous system of the primitive vertebrate and that outlying structures must have a different homology. This position is further strengthened by a study of neuromeres and sense organs, as will presently appear. It will be shown to have an important bearing on the conception of the structure of the primitive vertebrate head.

Another important event which occurs in this stage is the formation of a new groove, the neural groove, in the region first occupied by the germinal depressions. This groove can readily be distinguished from the posterior germinal depression by the fact that the latter at this time has either entirely disappeared or is fast degenerating while the new groove is deeper and sharper than the old grooves and is much narrower than the posterior depression (ng, fig. 2, D). In Plate I, figs. 4, 5, 6, the posterior

germinal depression is in various stages of degeneration, while in Plate I, fig. 7, the sharply defined neural groove is seen, being especially prominent in the posterior half of the neural plate. Photographs of succeeding stages, all except Plate I, figs. 8, 9, show the neural groove. The anterior germinal depression persists as a long narrow pit near the anterior end of the neural groove but not extending exactly to the end of the groove (agd, fig. 2, F). The neural groove is seen in but a small proportion of the eggs of this stage but after stage 7 is present in all the eggs and persists to the closing of the neural canal. It may properly be called a neural groove. While the two grooves called the germinal depressions appear before there is the slightest indication of a nervous system and begin to degenerate before the anlage of the nervous system is clearly defined, this third groove appears as the neural plate is forming and persists even in the fully formed neural canal. It is the only groove the history of which is closely identified with that of the nervous system.

The germinal depressions present in this stage show no new features. The anterior depression in the majority of eggs is short and deep and clearly marked (agd, fig. 2, A, C; Plate I, figs. 4, 5, 6), but in a few eggs it is impossible to make the distinction between the anterior depression and the posterior depression or neural groove. The posterior depression, as in stage 4, is either absent (fig. 2, A) or is very wide and shallow (fig. 2, B, C, E).

The transverse cephalic groove has now extended in a few eggs until it entirely cuts off the procephalic lobes (tcg, fig. 2, A, Plate I, figs. 6, 7). This groove shows considerable variation in length and width as the figures show. Moreover, it may extend clear across one-half of the neural plate before it shows at all on the other half (tcg, fig. 2, C; Plate I, fig. 6). In the latter case the embryo usually shows other signs of more rapid development on that side where the groove is present. The infundibular depression (id, fig. 2, C), for example, may show on one side only or in later stages the neuromeres may develop faster on one side than the other.



The infundibular depression in a majority of cases (id, fig. 2, C, F,) begins to form where the neural groove meets the peripheral groove, but when it appears before the formation of the neural groove it lies in front of the anterior germinal depression. In later stages the infundibular depression becomes a very important landmark on the procephalic lobes.

The blastopore, the transformation of which goes on more or less independently in point of time compared with the transformation of the anlage of the central nervous system, has now in a majority of the eggs given rise to three important structures, the blastogroove, the anus and the neurenteric canal. The circular blastopore becomes an oval (bp, fig. 1, A, B) then narrows into a slit (bg, fig. 1, C) and since the walls of the slit in reality touch each other the slit becomes a groove, the blastogroove. The length of the groove is approximately the diameter of the original blastopore. In some cases it may seem to be a little shorter, probably because there is a slight concrescence of the walls of the groove or slit at one or both ends. This account agrees with the description given by Jordan ('95), Eycleshymer ('93) and Morgan ('97) for various Amphibia. The statement of these authors, however, that the anus in *Salamandra* is a persisting part of the blastopore needs confirmation. It is maintained by Morgan that in the *Anura* the anus is a new structure and in *Amblystoma* a careful study of this region leads to the same conclusion. At this stage a posterior extension to the blastogroove has appeared carrying the slit backward until it touches the groove which in earlier stages bounds the blastoporic lip (bg, fig. 2, B, fig. 2, B, a). This backward extension of the blastogroove is the anus. In other words the anus in *Amblystoma* forms directly behind the blastogroove and in connection with it, so that in appearance it is the posterior end of the groove, there being no distinct boundary between the two. This forms a condition between that of the frog, where the anus develops separate from the blastogroove, and that of the newt, where if Morgan's account is correct the anus is a persisting part of the blastopore. At the anterior end of the blastogroove there remains a small pore, the neurenteric canal (nec, fig. 2, B).



A large part of the eggs as Eycleshymer has observed show no sign of this canal. His inference, however, that the canal is never formed in those eggs in which it is not seen does not follow necessarily, for like the transitory structures already described it may appear and disappear very quickly. Eycleshymer's statement could be proved only by following through the individual development of a single egg. In the absense of such evidence it is logical to conclude that the neurenteric canal, like the posterior germinal depression and the neuromeres, is of very general occurrence. The growth in length of the embryo which becomes apparent in this stage involves the question of whether or not the blastogroove grows in length. This has been a matter of dispute. Semon ('01) for *Ceratodus* has given perhaps the most detailed and careful description of a growth in length of the blastogroove. The earlier writers, Miss Johnson ('84) and Schultze ('88), have confused the blastogroove with other grooves and therefore their description of an elongating groove hardly affords any light on this problem. Jordan ('93) distinctly states that the blastogroove of the newt does not exceed in length the diameter of the blastopore. Morgan ('97) and Eycleshymer ('95) have not described any elongation in the various forms of *Amphibia* which they have studied. An elongation of the blastogroove in *Amblystoma* is evident after the disappearance of the neurenteric canal (bg, fig. 2, B). This is a result of the general elongation of the region in which the groove lies. The neural plate, too, elongates rapidly in this region as can be seen by comparing drawings A and B in fig. 2, noting in each case the length of the procephalic lobes as compared with the entire length of the plate. Such an elongation carries back the posterior end of the blastogroove and the anus. There is, however, no growing forward of the blastogroove since its anterior end does not approach the transverse cephalic groove but rather each end is slowly receding from the transverse groove. Now that the history of all four of these grooves has been traced, a more detailed comparison may be made by means of transverse sections. Fig. 3, A shows the anterior germinal depression in its average condition as regards depth of groove, while fig.

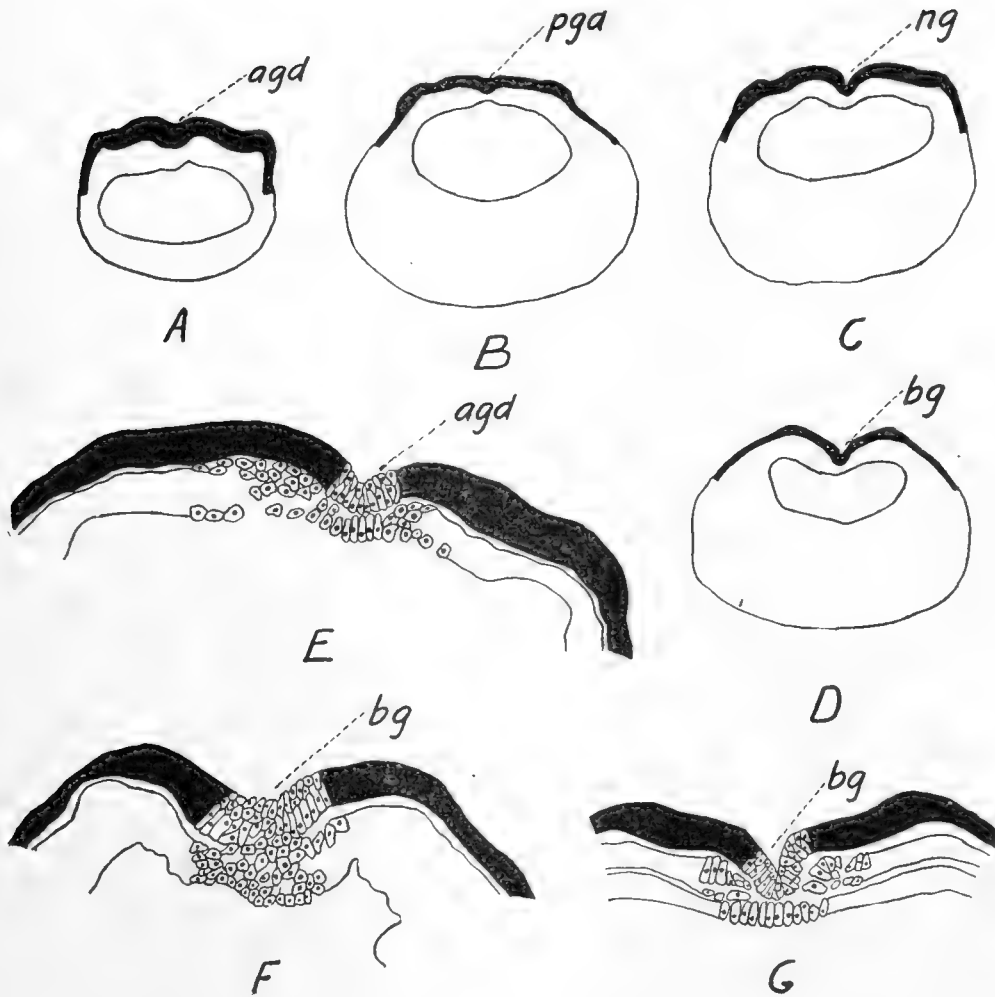


FIG. 3. Transverse sections of the open neural plate. E, F, G are highly magnified to show the cell structure. agd, anterior germinal depression; bg, blastogroove; ng, neural groove; pgd, posterior germinal depression.

3, B shows the posterior depression of the same embryo. The difference between the two grooves is a difference of depth. Fig. 3, C, a section of a slightly older embryo, shows the much more sharply defined neural groove as it appears in the posterior half of the neural plate. The walls of this groove form a more acute angle than the walls of the posterior depression and the depth of the groove is decidedly greater. The anterior end of the blastogroove, shown in fig. 3, D, has about the same depth as the neural groove but it will be noticed that the walls of the blastogroove form a sharp angle with the surface of the neural

plate, a condition very different from that shown in fig. 3, C. These differences, although not very great, go to support the position maintained in this paper that four different grooves appear in the median dorsal line of the embryo of *Amblystoma*.

Some account should also be given here of the histology of the grooves. It has been assumed by many writers that there is one primitive groove running over the dorsal surface of the embryo showing throughout its length a fusion of the germ layers. Miss Johnson ('84), however, has found that in the newt there is a fusion of germ layers only in the vicinity of the closed blastopore and that there is an "apparent fusion" in the "anterior pit" as she calls the deeper portion of the anterior germinal depression. In *Amblystoma* the ectoderm and endoderm are clearly separated in the region of the neural groove and of the posterior depression out the condition in the anterior depression and in the blastogroove is different. At the deepest part of the anterior germinal depression the ectoderm is pressed down against the endoderm (fig. 3, E). The fusion, however, is not real but merely "apparent" or mechanical for there is a readily recognizable difference in shape and contents between the two types of cells, and there are no cells between the two layers intermediate in character. In the case of the blastogroove there is a real fusion of layers (fig. 3, F). At the posterior end of the groove the cells can be followed through from the ectoderm to the endoderm. There is no space between the two layers, and the cells lying midway between the two are intermediate in character. Toward the anterior end of the blastogroove, however, the anlage of the notocord has differentiated and the two germ layers have separated (fig. 3, G). A few small undifferentiated cells may still be seen in the bottom of the anterior end of the blastogroove resembling those at the posterior end.

The peripheral groove and neural crest in this stage are extending backward and meet around the posterior end of the neural plate (fig. 2, A, B). This process clearly defines the posterior limit of the neural plate and it is seen that the anus lies outside the neural plate in the region of the neural crest (a, fig. 2, B).

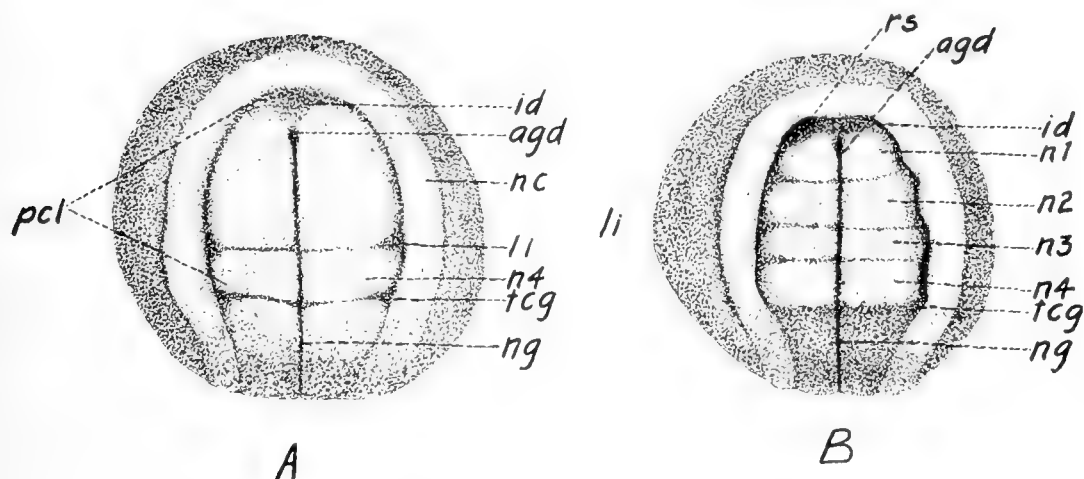


FIG. 4. Surface views of entire embryos to show the development of neuromeres, sixth stage. agd, anterior germinal depression; id, infundibular depression; li, lateral infolding; n1, n2, n3, n4, the four neuromeres of the procephalic lobes; nc, neural crest; ng, neural groove; pcl, procephalic lobes; rs, retinal spot; tcg, transverse cephalic groove.

Stage 6 (Fig. 4, 5; Plate I, figs. 8-11). The following description of the development of the neuromeres in the neural plate should perhaps be prefaced by a few statements showing the evidence upon which it is based. The drawings are all made from specimens killed and hardened in chromic acid but the number and arrangement of the neuromeres has been confirmed by a study of living eggs, as well as by eggs killed in other fluids and by longitudinal sections. Four is the largest number of neuromeres found in the procephalic lobes. Where there are less than four there is always apparently room for the development of the full number.

The first neuromere to appear lies just in front of the transverse cephalic groove (n4, fig. 4, A). Its anterior boundary is formed like the transverse cephalic groove from a pair of pits which develop in the peripheral groove and which in a few eggs are visible in earlier stages (fig. 2, A). In this stage the groove is seen in various degrees of development. It is nearly complete in fig. 4, A and fully formed in fig. 5, A. The three remaining neuromeres appear in the same way but do not follow any definite order in their appearance. Fig. 4, A and B, represent

typical eggs showing the first and last steps in the process, while some of the intermediate steps are shown in fig. 5. Plate I, figs 8 and 9 show the faint appearance of one neuromere. These two embryos are especially valuable because they show, although faintly, the anterior and posterior germinal depressions and hence it is possible to locate the neuromere exactly. It lies opposite the posterior end of the anterior depression as can be seen plainly in Plate I, fig. 9. Plate I, fig. 10 shows all four neuromeres. The first and most anterior one is the faintest and is rather difficult to see in a photograph. Plate I, fig. 11 shows the neuromeres as seen from the side. Their shaded posterior margins appear as dark bands, the first neuromere being very faintly marked off from the second. While it is very difficult to show the details in photographs they are offered as a general confirmation of the more exact history of the neuromeres as shown by drawings. This follows the convincing method used by Locy ('95) in his treatment of the subject of neuromeres in the embryo of *Acanthias*.

This brief history of the development of the important procephalic neuromeres should be supplemented by a description of some details of minor importance. The neuromeres are usually not all of equal size. The fourth neuromere, the first to appear in point of time, is often very prominent (n4, fig. 4, B, fig. 5, D) and the second, although never higher than the others is sometimes much wider (n2, fig. 4, B, fig. 5, D). The first neuromere is usually the most faintly marked of all. Variations from the typical pattern are common particularly in the direction of so called "hemi-embryos" (fig. 5, B, E). Neal ('98) has used this fact of lack of correspondence between right and left sides as an argument against Locy's theory of neuromeres. Such a condition, at least in *Amblystoma*, seems to show rather that the right and left sides develop more or less independently. After the full development of the neuromeres there is an entire correspondence between the two sides as will be shown in the next stage. Very rarely there are indications of more than the usual number of neuromeres. Among the hundreds of eggs examined there were only two such specimens found one of

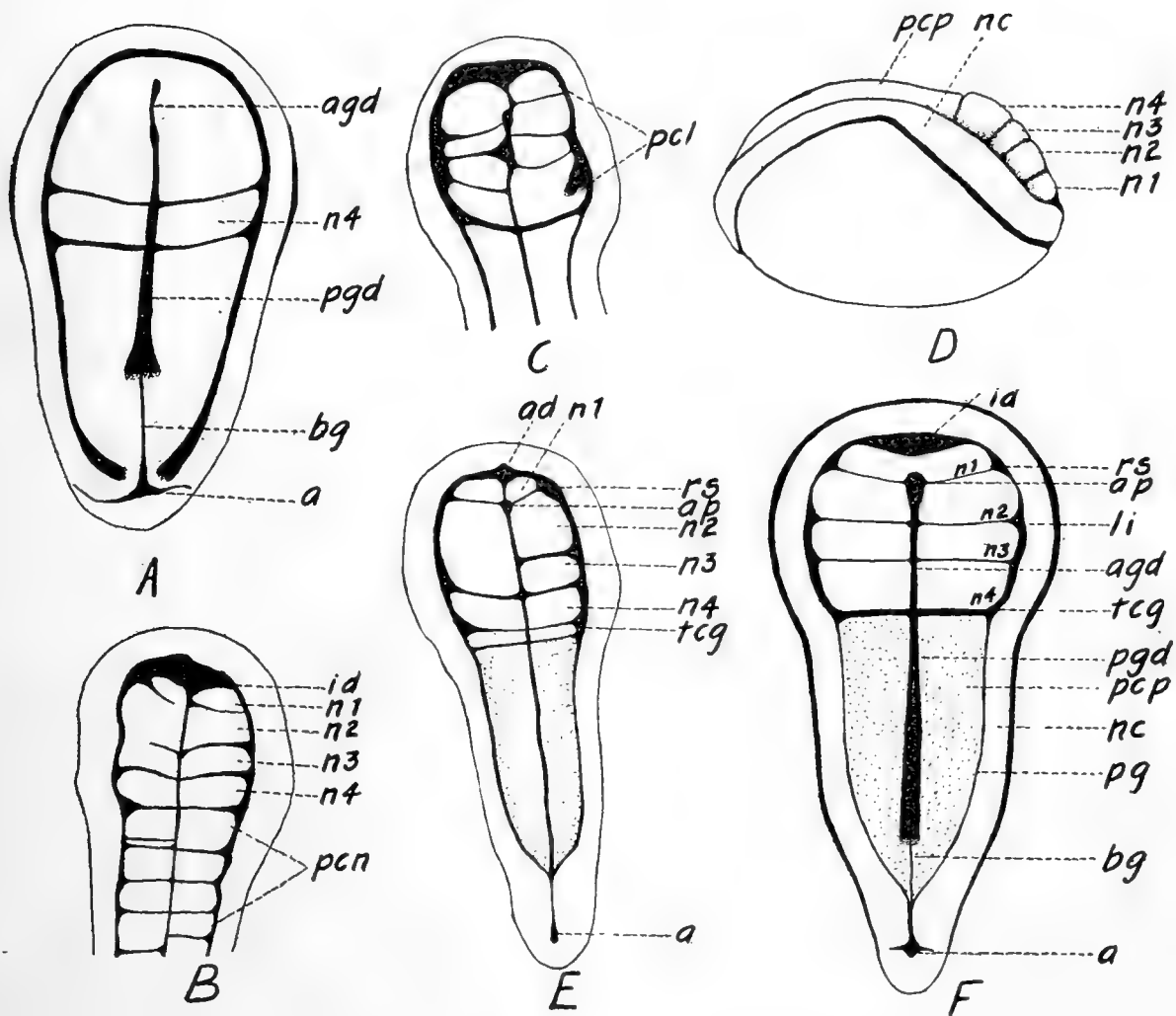


FIG. 5. Neuromeres in various stages of development. F is a diagram showing all the features of the fully developed neural plate. a, anus; ad, anterior depression; agd, anterior germinal depression; ap, anterior pit; bq, blastogroove; id, infundibular depression; li, lateral infolding; n1, n2, n3, n4, four neuromeres of the procephalic lobes; nc, neural crest; pcl, procephalic lobes; pcn, postcephalic neuromeres; pcp, postcephalic plate; pg, peripheral groove; pgd, posterior germinal depression; rs, retinal spot; tcg, transverse cephalic groove.

which is shown in fig. 4, C. The irregularity in the appearance of the neuromeres suggests abnormality especially since the cases are so very rare. It seems a fair conclusion that the variations mentioned in this paragraph in respect to size of neuromeres, degree of development of right and left sides, and number of neuromeres are only such as one might reasonably expect to find in working over material of this nature.

This group of four neuromeres constituting the procephalic lobes is further marked off from the rest of the plate by its height (fig. 5, D; Plate I, figs. 11, 12), and also by its darker color as in previous stages. These distinctive characters make it convenient to apply the term "tagma" suggested by Lankester for a more or less isolated and independent group of segments. The aptness of this term will become more apparent as the development of the procephalic lobes is followed in the succeeding stages and when it is discussed in the conclusion.

It has already been noted in this paper that there are few observations of neuromeres present in the open neural plate. Kupffer ('93) found six or seven neuromeres in the expanded end of the plate of *Salamandra atra*. Froriep ('91, '93,) found in the same region of Triton five neuromeres and in *Salamandra* four, but he later denied their real metameric value. Locy ('95) found a few large divisions in the cephalic plate of *Amblystoma* and in the plate of *Rana palustris* which, however, he did not regard as true neuromeres. Eycleshymer ('95) found divisions in the neural plate of *Amblystoma* which he regarded as artifacts. Hill ('00) found in the anlage of the combined forebrain and mid-brain of the trout and the chick five neuromeres. Although these authors differ as to the number of segments that shall be assigned to the brain region of the neural plate and although they fail to agree as to the meaning of the segments, yet the evidence taken as a whole is strongly in favor of the presence of true neuromeres in the open neural plate. The cause of disagreement, particularly as to the number of segments, seems to be the lack of landmarks. The broad anterior end of the neural plate cannot be traced directly into a well defined region of the brain without some such boundary as the transverse cephalic groove. Kupffer admitted that he was unable to find such a landmark in *Salamandra*. Locy used the entire expanded portion of the plate as forming in a general way the anlage of the brain. Hill is the only author who has yet described anything corresponding to the transverse cephalic groove of *Amblystoma*. He found that in the solid anlage of the trout brain there were two deep "dorsal grooves" one in

front of the mid-brain and one behind the cerebellum, and in the closed tube of the chick the constrictions in front of and behind the mid-brain are deeper than the others. The groove behind the cerebellum in the trout may possibly be homologous with the transverse cephalic groove of Amblystoma, but the peculiarities of the formation of the brain of the former make such comparisons of little value. These observations, then, while they furnish proof of the actual segmentation of the neural plate, emphasise particularly the great importance of the presence of such a land mark as the transverse cephalic groove of Amblystoma.

Scarcely less important than the neuromeres are the anlagen of the lateral eyes. These appear as a pair of oval pigmented depressions lying between the neural plate and the neural crest (rs, fig. 4, B, fig. 5, E). In Amblystoma, although the retinal spot is unquestionably present in a small proportion of the embryos, it is not nearly so prominent as in *Necturus* and *Rana palustris*. In the last named form it is very prominent in all eggs of the open neural plate stage, occupying exactly the same position as in Amblystoma between the neural plate and the crest.

In the review of the literature on this subject it was shown that the first observers of this retinal spot Whitman, Eycleshymer, Locy, Hill were not specific as to its exact position in relation to the edge of the neural plate. They have described the spots as being located in a general way on the plate, not beside it. In Amblystoma the retinal spots are very clearly located just lateral to the neural plate in the peripheral groove. If the proposition that the neural plate represents the ancestral brain be granted, then the eyes were located not on or in the brain, as some authors have claimed, but just lateral to it in a position corresponding to that found in some of the higher invertebrates. A careful consideration of this question of the position of the retinal spots is important in the understanding of the history which follows in succeeding stages.

As the neuromeres form on the procephalic lobes the neural crests in this region become scalloped on their inner surfaces



to correspond to the segments of the neural plate (fig. 4, B), but the crest on the upper surface and on the outer edge is smooth except in a very few cases where some of the grooves between the neuromeres are extended laterally clear across the crest. This description differs from that of Locy ('95) who has pictured for *Amblystoma* not only a few large neuromeres in the open neural plate but also a beaded appearance in the neural crests. This beaded appearance was not apparent in any of the embryos of this stage used in the preparation of this paper.

The neural crests in this stage begin to move in toward the median line. The change is seen first at the posterior end of the neural plate where the two crests meet just in front of the anus (fig. 5, E). The anus which is now dorsal in position soon moves to a ventral position and loses all connection with the neural crests. In this respect *Amblystoma* seems to agree exactly with the condition which Morgan ('97) has described in detail for *Rana*. One point in addition should be noted. The infoldings which in the earlier stages extended for a short distance to the right and the left of the anus now fade away and disappear as can be seen by a comparison of A and E in fig. 5. Further changes in connection with the moving in of the neural crests will be described as they are pictured in connection with older stages.

The neural groove, the origin of which has already been traced, is easily recognized in all embryos (fig. 4, A, B). The posterior germinal depression with which the neural groove might be confused has now disappeared except in a very few eggs and the only remaining part of the anterior depression is the deep pit which has been shown in the preceding stage. The blastogroove disappears from view as the neural crests close over and in a majority of eggs it can no longer be distinguished from the neural groove (fig. 5, E). This condition of the various grooves persists until the neural canal is completely closed.

The open neural plate with its various structures, grooves, crests, neuromeres, etc., has now reached its maximum development. Fig. 5, F, is a diagram illustrating the different structures, all of which appear in every egg in the course of its develop-

ment although a single egg is rarely found which shows them clearly all at one time as they are shown in the diagram. The two halves of the plate for example often develop unequally and, moreover, the order of appearance and disappearance of the various structures varies greatly in different eggs, and again there are a few transitory structures which in some eggs may never be prominently developed. The confusion caused by these three factors has been carefully considered in the description of the preceding stages. It is evident that there are a few important general factors underlying the course of the development of the neural plate; the markings on the surface of the embryo take the form of grooves and ridges; these grooves and ridges run transversely and longitudinally marking off transverse and longitudinal zones. Such a conception of zones will be made clearer by the following review of the various stages in the development of the plate.

The embryonic area is first divided longitudinally by a series of grooves, the anterior germinal depression (agd, fig. 5, F), the posterior germinal depression (pgd), and the blastogroove (bg), all three of which develop in the median line of the embryo in the order named. Then to the right and left of the median line develop the low wide ridges of the neural plate which is bounded by the peripheral groove (pg). Outside of the peripheral groove another ridge, higher and narrower, develops to form the neural crest (nc). Later the first three grooves disappear, except the anterior portion of the anterior germinal depression, and their place is taken by the neural groove which is not shown in the diagram. The transverse zones appear after the longitudinal zones. First the groove which is called the transverse cephalic groove (tcg) divides the procephalic lobes from the rest of the plate, then the fourth neuromere (n4) is formed, then the first three neuromeres appear, not following any regular order. In a few eggs of later stages neuromeres appear behind the first four of the procephalic lobes but their history cannot be traced in Amblystoma nor even their number and arrangement determined. In this list the blastopore, the retinal spot (rs), the lateral infolding (li) and the infundibular

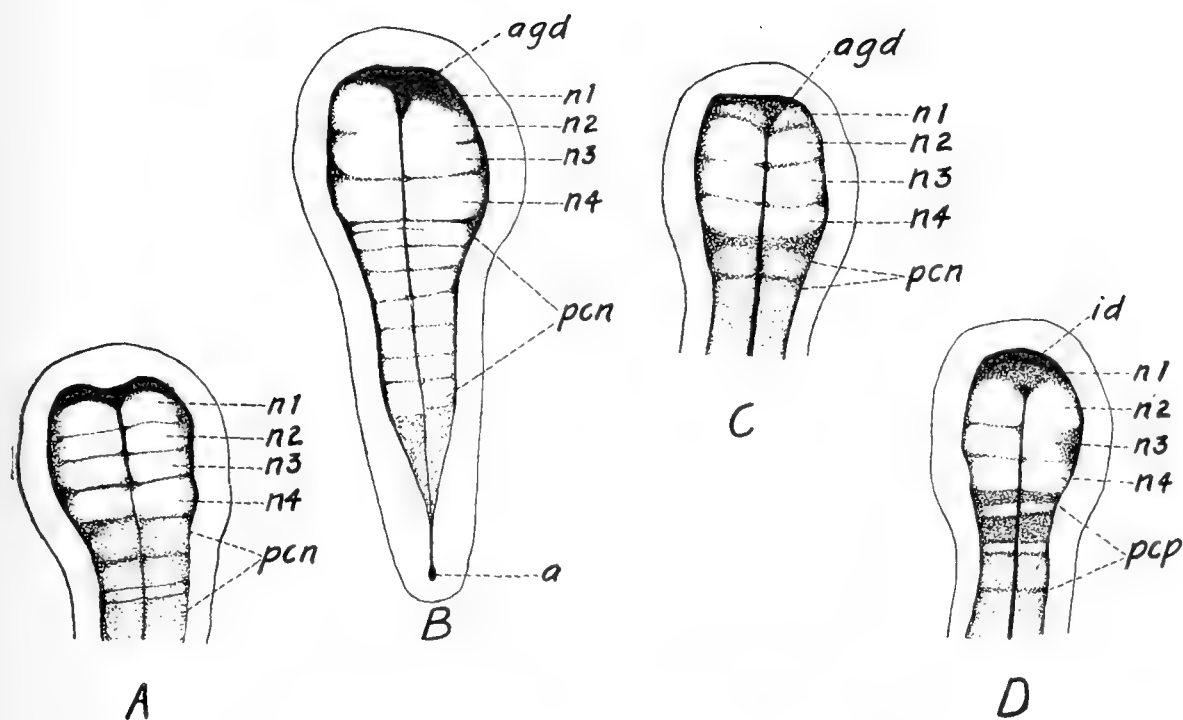
depression (id) have been omitted. The retinal spots may as Locy has claimed be a part of a longitudinal series of sensory patches but *Amblystoma* shows only the first pair unless the lateral infoldings have some such significance. In any case both the retinal spots and the lateral infoldings lie in the peripheral groove. The blastopore after it has been transformed into the blastogroove also takes its place among the longitudinal zones. The infundibular depression in its later development includes the whole of the first neuromere and so marks that transverse division to a certain extent as standing apart from the other neuromeres.

The following table gives concisely the arrangement of the structures mentioned above.

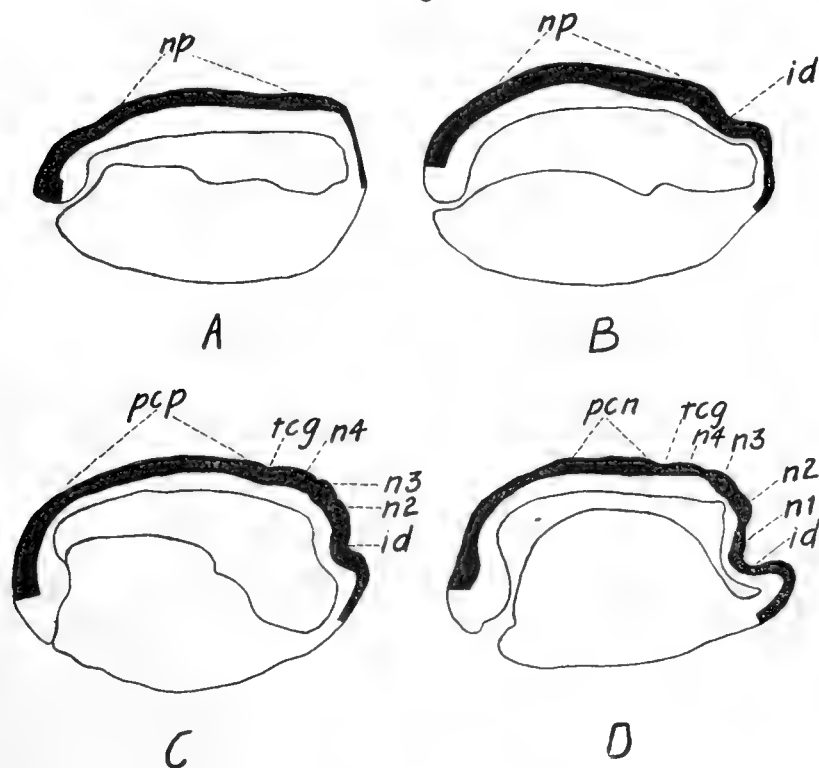
<i>A. Longitudinal Divisions</i>	<i>B. Transverse Divisions</i>
<ol style="list-style-type: none"> <li>1. posterior germinal depression.</li> <li>2. anterior germinal depression.</li> <li>3. blastogroove, developing from the blastopore.</li> <li>4. neural plate.</li> <li>5. peripheral groove, with lateral infoldings and retinal spots.</li> <li>6. neural crests.</li> <li>7. neural groove, which forms where the two germinal depressions and the blastogroove wholly or in part disappear.</li> </ol>	<ol style="list-style-type: none"> <li>1. procephalic lobes, a tagma marked off by the transverse cephalic groove.</li> <li>2. fourth neuromere.</li> <li>3. first three neuromeres, the first laterfolding down as the infundibular depression.</li> <li>4. neuromeres of medulla and spinal cord, a region where tagmata cannot be observed in <i>Amblystoma</i>.</li> </ol>

Stage 7 (Fig. 6, 7; plate I, figs. 12-15). It has already been shown that in front of the procephalic lobes the peripheral groove becomes deeper and wider forming a very conspicuous depression, the infundibular depression (id, fig. 6, D). This grows deeper and wider in older embryos until finally the entire first neuromere is folded down and lies on the floor of the depression (n1, fig. 6, C) where it remains permanently at a level considerably lower than that of the other neuromeres.

The whole process takes place very quickly with the result that a very few embryos show intermediate stages. Nearly all are in the condition shown in plate I, figs. 12-15 where three very regular neuromeres are visible in the procephalic lobes,



6



7

FIG. 6. Neural plates of the seventh stage showing the downfolding of the first neuromere and the development of neuromeres behind the procephalic lobes. a, anus; agd, anterior germinal depression; id, infundibular depression; n1, n2, n3, n4, neuromeres of the procephalic lobes; pcn, postcephalic neuromeres; pcn, postcephalic plate.

FIG. 7. Longitudinal sections cut a little to one side of the median line showing development of neuromeres and infundibular depression. id, infundibular depression; n1, n2, n3, n4, neuromeres of the procephalic lobes; np, neural plate; pcn, postcephalic neuromeres; pcn, postcephalic plate; tcg, transverse cephalic groove.

the remaining one being entirely hidden from view in a deep furrow at the anterior end of the neural plate. The sections illustrated in fig. 7 show the process of infolding. In section A there is not yet any appearance of the infundibular depression. The three succeeding sections show the origin and development of the depression and the flexure that is produced in the neural plate by the infolding. The neuromeres cannot be readily observed in sections A and B but in C and D the raised procephalic lobes are easily recognized and in the latter it is possible to identify the neuromeres particularly the fourth. The relation of the infundibular depression to the neuromeres will be shown again in the next stage.

This infolding involves also the anterior pit which represents the original anterior germinal depression (agd, fig. 6, C). The pit may be seen for a short time lying on the floor of the infundibular depression but it soon disappears and it cannot be seen at all in embryos after the closing of the neural canal, even by a careful dissection of the brain to uncover this region. The infundibular depression itself, however, persists as a very important landmark.

Kupffer ('04) has given a very good account of this region of the embryonic brain, the hypencephalon, as he calls it, in a series of vertebrates. According to him it is bounded behind by a transverse ridge, evidently the anterior edge of the first neuromere as seen in *Amblystoma*, which he calls the tuberculum posterior, but in front in the earlier stages there is no definite boundary. He observes that the infundibulum develops from the posterior ventral part of the hypencephalon while the optic chiasma forms in front of the infundibulum on the floor of the hypencephalon. It should be noted that he failed entirely to trace the neuromeres of the open neural plate into this stage. The determination of the exact relation of the first neuromere to this infundibular depression would certainly form an important contribution to the history of this region.

Behind the procephalic lobes there appears a new series of neuromeres, the postcephalic neuromeres (pcn, fig. 6), which apparently develop in order from before backward. They

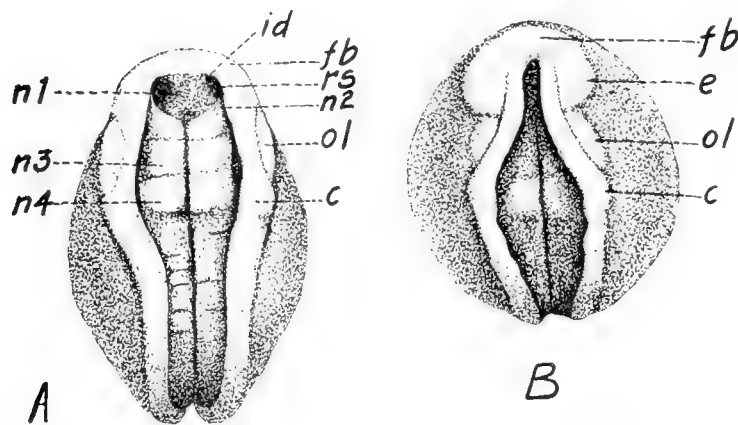


FIG. 8. Entire embryos showing the first steps in the closing of the neural canal, and the relation of the neuromeres to the developing cerebral vesicles, stage eight. c, cerebellar crest; e, eye; fb, fore-brain; id, infundibular depression; n1, n2, n3, n4, neuromeres of the procephalic lobe; ol, optic lobe; rs, retinal spot.

are usually indistinct and in at least half the eggs of this stage they cannot be seen at all. Fig. 6, B, illustrates the best specimen of the entire collection for this purpose, but other eggs vary both in number of the neuromeres and the appearance of the neuromeres. In examining the four embryos shown in fig. 6, it will be noticed that a few narrow neuromeres are present. While such narrow neuromeres appear only rarely, when they do appear they always alternate with the usual wide neuromeres and there are no neuromeres intermediate in size between the two forms.

It might be expected that these postcephalic neuromeres would be found in groups or tagmata like the procephalic neuromeres. Thus we can speak theoretically of a metencephalic tagma, the anlage of the medulla, and of a spinal cord tagma, the anlage of the spinal cord, but in *Amblystoma* the postcephalic neuromeres are too rudimentary and transitory to discover any such relations.

As the neural crest closes over the plate all signs of segmentation behind the procephalic lobes disappear. No landmarks are left that can be followed into the adult brain.

Stage 8 (Figs. 8, 9, 10, 11). In this stage the closing of the neural tube is completed. The crests meet first in front of the

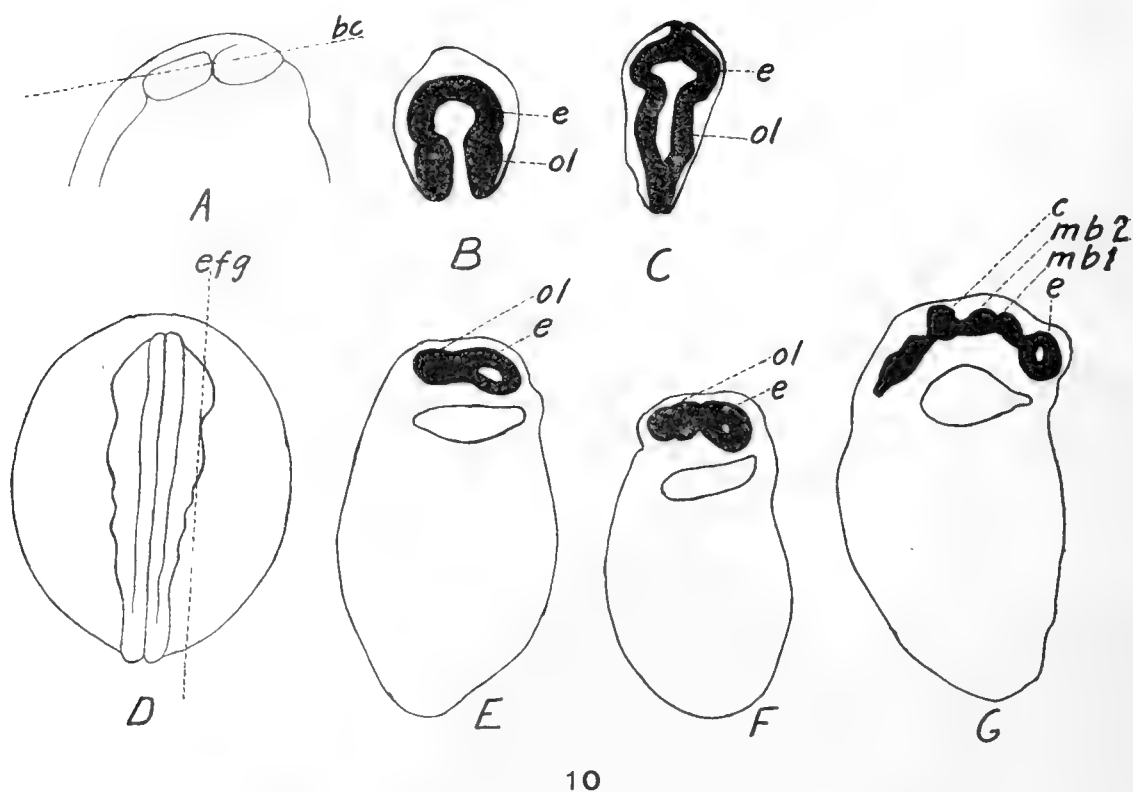
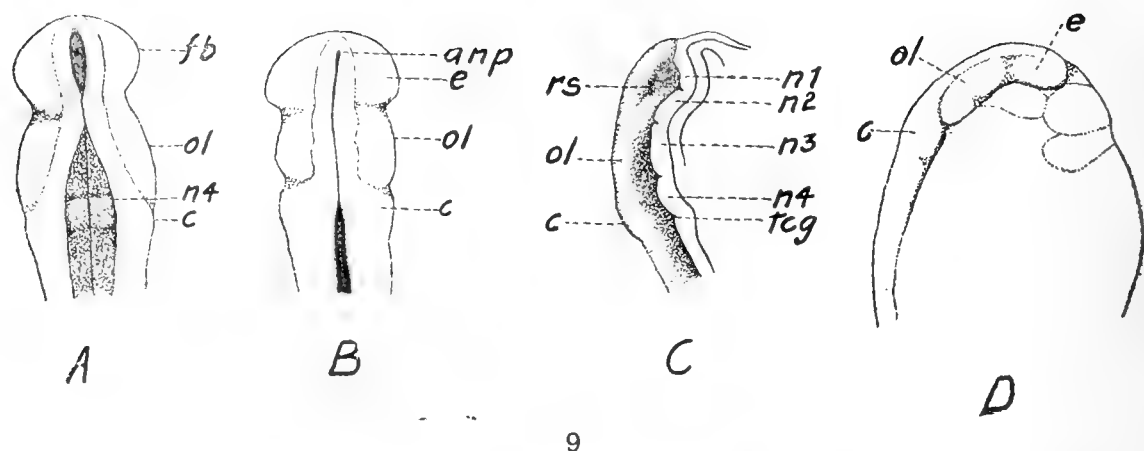


FIG. 9. Later stages in the closing of the neural canal and the development of the cerebral vesicles. C is a view of the inside of a halved neural tube, and D is a view from the side of an entire embryo. anp, anterior neuropore; c, cerebellar crest; e, eye; fb, fore-brain; n1, n2, n3, n4, neuromeres of the procephalic lobes; ol, optic lobe; rs, retinal spot; tcg, transverse cephalic groove.

FIG. 10. Sections to show the structure of the walls of the neural canal. The diagram A shows the location of sections B and C. C is an older embryo than B. The diagram D shows the location of sections E, F and G. F is an older embryo than E, and G is older than F. e, eye; c, cerebellar crest; mb1, mb2, divisions in the optic lobes or mid-brain; ol, optic lobes.

anus as described above, next they meet over the posterior part of the cephalic lobes and from these two points the process of closing goes rapidly on. The anterior end of the canal remains open for a brief period as the anterior neuropore (anp, fig. 9, B), but the neuropore does not lie at the extreme anterior end of the canal, for there is a slight concrescence of the ridges in front of the neuropore leaving a groove which is discernible in all eggs of the right condition, and furthermore the blind end of the neural tube always extends an appreciable distance in front of the neuropore. These relations may be easily seen in the specimens shown in figs. 8 and 9.

This concrescence of the fold in front of the neuropore is an important factor in determining the anterior limit of the neural axis. Kupffer and other authors have considered the anterior border of the neuropore as the anterior end of the brain but Johnston ('05) has more recently shown that this point owing to a concrescence, such as has just been described, varies greatly in different types of vertebrates. Johnston's conclusion, however, that the anterior end of the brain from a morphological point of view lies at "that point at which the brain plate meets the general ectoderm at the same time that it comes in contact with the anterior end of the endoderm" does not form a criterion that can be applied to the conditions found in Amblystoma, for the neural plate does not come in contact with the general ectoderm since the neural crest intervenes (fig. 8, A). If the open segmented neural plate be considered as the neural axis, or the anlage of the neural axis, then the anterior margin of the first neuromere constitutes the anterior limit of the nervous system, a point, however, which cannot be traced into the adult brain of Amblystoma although it is visible just before the closing of the neural tube. On the other hand, if the anterior border of the neural crest be taken as the point in question, on the ground that the neural crest forms an integral part of the central nervous system, at least in modern vertebrates, then the neural axis ends at a point slightly in front of the neuropore, a point which in Amblystoma can no more be determined with accuracy in adult stages than the anterior limit of the neural plate. Thus Amblystoma



although it shows the weakness of the old theories does not present any exact point that may safely be taken as the anterior limit of the adult brain.

Before going further into a discussion of the neuromeres of this stage it will be necessary to describe the origin of the landmarks of the adult brain. As soon as the neural crests begin to close a constriction appears in the neural canal marking off the primary fore-brain vesicle (fb, fig. 8). Then a second constriction appears marking off the mid-brain vesicle (ol, fig. 8). When once these landmarks have been identified they may be readily traced back to embryos as young as those shown in fig. 8, but they cannot be seen in any younger specimens. This process of the development of the two primary brain vesicles becomes very clear by tracing the series backward in the following order—fig. 9 B, fig. 9 A, fig. 8 B, fig. 8 A. A third landmark, the first division or segment of the hind-brain vesicle, appears in the neural crest (c, fig. 8.) In its later stages (c, fig. 9) it appears as the first hind-brain neuromere or cerebellar neuromere of the closed neural tube. The cerebellum arises from its roof.

The relation of the neuromeres to these three well recognized landmarks is clearly a matter of prime importance but its importance may be overestimated as compared with the relation of the whole group or tagma of procephalic neuromeres to the parts of the adult brain. This tagma has been shown to be older than the individual neuromeres and to be more prominent and regular in its appearance. The first problem is to determine the relation of the tagma as a whole to the primary brain vesicles; the relation of the individual neuromeres to these vesicles or to other later landmarks may prove to be a different and more difficult problem. It is possible that in *Amblystoma* in which the segmentation of the neural plate is somewhat rudimentary the original number of neuromeres in each tagma may be subsequently changed by fusion, or by temporary suppression. Be that as it may, it is better to consider first the relation of the procephalic lobes as a whole to the adult brain.

The posterior boundary to these lobes has been shown to be the great transverse cephalic groove, the first landmark to appear

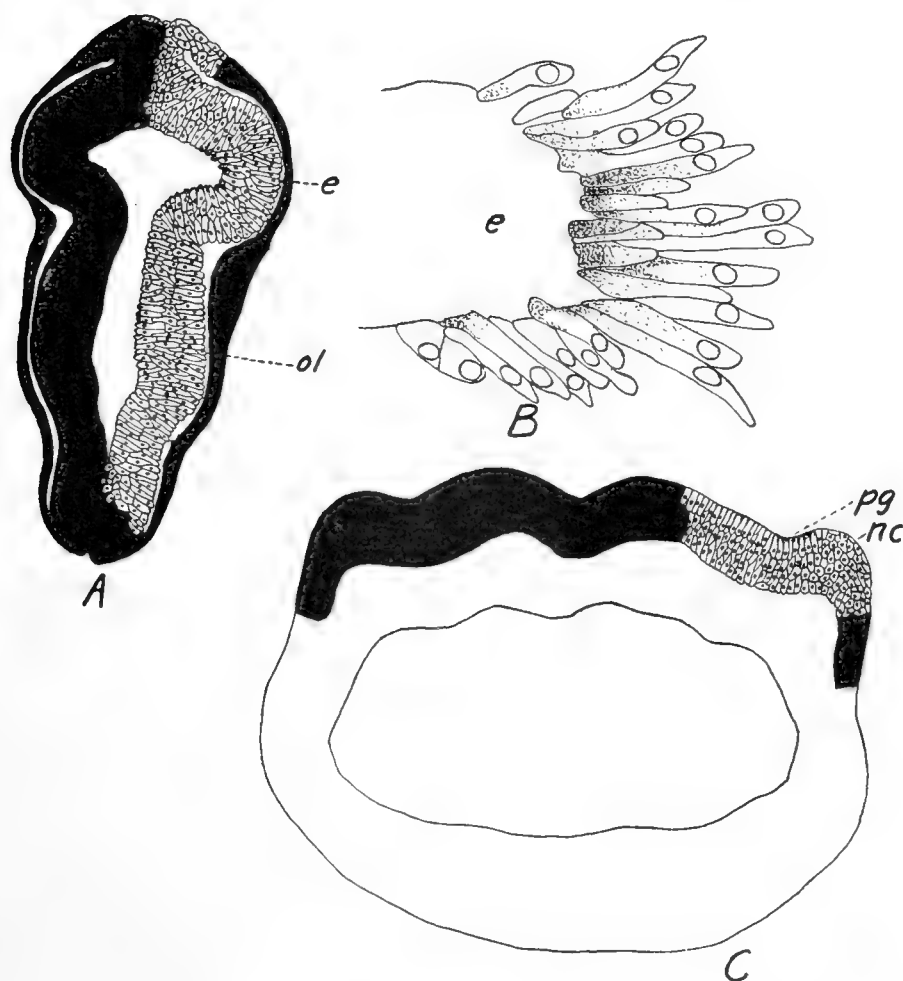


FIG. 11. A is a section through the eyes and optic lobes, B is a portion of the inner wall of the eye shown in A greatly enlarged to give the cell structure, C is a portion of the open neural plate seen in cross section showing the structure of the neural crest and peripheral groove. e, eye; nc, neural crest; ol, optic lobe; pg, peripheral groove.

on the surface of the neural plate. An examination of the surface views of the stage in which the primary brain vesicles are beginning to appear shows this groove lying just behind the cerebellar swelling of the neural crest (fig. 8). An embryo that has been split in halves shows this relation to better effect (fig. 9, C). Behind the fourth neuromere is the transverse cephalic groove (tcg). Directly above the fourth neuromere is the cerebellar crest (c.) A careful examination of Plate 1, fig. 15, shows the cerebellar crest and its relation to the posterior boundary of the procephalic lobes exactly as in the figures just described. The

evidence, therefore, seems fairly conclusive that the region of the procephalic lobes includes the fore-brain, mid-brain and that part of the hind-brain in which the anlage of the cerebellum is found.

The determination of the relation of the individual neuromeres to landmarks of the adult brain is a more difficult problem. It should be borne in mind that this is the last stage in which it is possible to identify the neuromeres with certainty since they very quickly disappear as the neural crests close over into the neural tube. The only trustworthy evidence then must come from the embryos of this stage. It will be best to consider the neuromeres separately beginning with the first or most anterior. This neuromere very clearly lies on the floor of the fore-brain vesicle (n1, fig. 8, A, fig. 9, C). A small portion of the second neuromere is bent down with the first until it may perhaps be considered to be involved with the infolding (n2, fig. 9, C). Johnston's ('05) statement that "the fore-brain is formed by a relatively enormous growth of the first neuromere especially of its dorsal part" seems to go considerably beyond the facts as they appear in *Amblystoma*. All that can actually be observed here is that the first neuromere forms the floor of the infundibular depression which at this stage constitutes the ventral portion of the primary fore-brain vesicle. That the cells of the first neuromere actually contribute to the formation of the sides and roof of the adult fore-brain may be very probable but other structures may very likely be involved also. The second and third neuromeres in the same way form the floor of the primary mid-brain vesicle (n2, n3, fig. 8 A, fig. 9, C). The second neuromere as has been stated sometimes appears to extend slightly into the fore-brain region, but the third lies wholly in the mid-brain. The fourth neuromere covers a region on the floor of the hind-brain vesicle lying directly below the cerebellar crests (n4, fig. 9, A, C). All these various relations may be summed up by the statement that the first neuromere contributes to the formation of the fore-brain, the second and third to the formation of the mid-brain, and the fourth to the formation of the anterior part of the hind-brain. It should be borne in mind, however,

that other structures outside of the neural plate may have a part in the formation of the brain.

The position taken by Patten is that the vertebrate brain is built up of various elements, some of which lie morphologically outside of the primitive neural axis. This hypothesis has been very suggestive. Some attempt has been made in this paper to test it by tracing as far back as possible the very beginning of the various parts of the brain, and by discriminating very carefully between structures that belong primarily to the neural plate and those that do not belong to it. It has already been shown that the germinal depressions are probably not "neural" grooves. The history of the neural crest has shown that it also is evidently not an integral part of the neural plate since it makes its appearance only after the plate is well marked off, since it is separated from the plate by a distinct groove and since it is not segmented like the plate. It now remains to discuss in this connection the development of two still more important structures, the lateral eyes and the optic lobes.

The first appearance of the lateral eyes has already been described as a pair of pigment spots lying just lateral to the anterior part of the neural plate in the peripheral groove. As the neural crests are raised up to form the neural tube the retinal spots come to lie in the lateral walls of the primary fore-brain vesicle (rs, fig. 8, A). The walls of the brain immediately begin to bulge out to form the optic vesicle (e, fig. 8, B), the direction of growth being backward as well as outward, as may be seen by comparing the figures of this stage with those of the next stage. The optic vesicle is comparatively large, as in most vertebrates, and seems to form at this stage the whole lateral wall of the fore-brain vesicle.

The histology and histogenesis of the retinal spot in Amblystoma and other amphibians, *Rana palustris* for example, has been described in detail by Eycleshymer ('93). He found that in Amblystoma the pigment in the early stages is not so well developed as in *Rana palustris*, neither do the retinal cells show the early differentiation into the characteristic columnar form. My own observations confirm Eycleshymer's work. It seems

to be true that scattered groups of pigment-bearing cells are found everywhere in the lining layer of the neural canal but the cells of the retinal spot bear more pigment than is found in any other region. Section A, fig. 11, shows the retinal spot (e) drawn to a large scale and section B shows the pigment-bearing cells still more highly magnified. These cells are just beginning to assume a columnar form such as Eycleshymer has figured for relatively younger embryos of *Rana palustris* and other amphibians. This pigment is collected at the ends of the cells along the walls. At this stage then the anlage of the retina may be clearly identified in sections as well as in surface views.

Of perhaps still greater significance is the development of the optic lobes from a region lying lateral to the neural plate. A deep groove, the lateral infolding, has already been described as forming on each side of the procephalic lobes (li, fig. 4, A). As the neural crests fold over, these grooves disappear but at the same time in the same region between the neural crests and the neural plate appear a pair of low ridges or lobes (ol, fig. 8). Each lobe is bounded anteriorly by the optic vesicle and posteriorly by the cerebellar crest. At first rather indistinct in outline it soon becomes very prominent and clear cut (ol, fig. 9). As its growth continues it bulges backward as may be inferred from the pear-shaped form which it assumes (fig. 9, B, D). It may push back slightly under the cerebellar crest (fig. 9, D). It becomes bilobed as can be seen better in the next stage although the beginning is shown in fig. 9, D.

A study of sections confirms this account of the early appearance of the optic lobes. Fig. 10, B, is a section cut longitudinally through the fore-brain vesicle and optic lobes in a plane shown by the diagram, A. The optic lobes appear as thickened walls of the neural tube lying directly behind the retinal spots. The walls of this region are slightly thicker than those of the fore-brain vesicle and the diameter of the canal is considerably less, a condition already shown in fig. 9, C, a drawing of a halved embryo. Section C, fig. 10, is cut through the same plane as B but the embryo is a little older, as appears from the fact that the neural canal is now closed. In comparing the two sections

it is seen that the optic lobes have become thinner and also a little longer. The cavity, too, has become larger. This enlargement of the optic lobes is seen in surface views of fig. 9. Sections E, F, G are longitudinal sections cut in a plane at right angles to sections B and C. Their position is shown by the line in the diagram at D. Section E shows the optic lobe extending posteriorly from the optic vesicle very much as in B. In section F the lobe has become slightly divided into two parts and in section G it is very clearly divided (mb1, mb2). This last section shows also how closely the cerebellar crest is related to the general neural crest as has already been shown in fig. 9.

The histology of this region presents no very distinctive features as the histology of the optic vesicle showed none. Section C, fig. 11, shows the cells of the peripheral groove (pg), the region in which the optic lobes arise. The cells present the same appearance as those of the neural plate but they differ strikingly from the cubical cells of the adjacent neural crest. There is slightly more pigment in the peripheral groove than in the plate. Section A, fig. 11, shows the cell structure of the optic lobes at about the time of the closure of the neural canal. The cells are of the same general form as those of the optic vesicles but they contain less pigment. This early differentiation of the optic lobes, therefore, is a differentiation of outer form not of cell structure.

Thus not only may the anlage of the retina be located on the surface of the embryo while the neural plate is still open but also what appears to be its ganglion, the anlage of the later visual center in the mid-brain, may be located just behind the retinal anlage. It seems probable that the ancestors of the vertebrates possessed an eye and optic ganglion lateral to the brain on either side and that these organs were later infolded into the brain when the central nervous system was transformed into a closed canal. This theory offers a reasonable explanation of the fact that the mid-brain unlike the rest of the tube has at the very outset a thickened layer of nerve elements on the roof and sides, the tectum opticum. Indeed such an explanation has before been hinted at by those authors who have endeavored to

find some morphological reason for the peculiar character of the mid-brain. Johnston ('05) suggests that at the anterior end of the nervous system there is included in the brain an "area which at the level of the acustico-lateral anlage is supposed to be left out." It has certainly, however, never before been demonstrated that the peculiar structure, the optic lobe, lies originally external and lateral to the neural axis.

From this history of the anlage of the lateral eyes and their centers, the optic lobes, it might be expected that the anlage of the parietal eyes could also be found near the open neural plate. Locy ('95) has claimed to have found them in the embryos *Squalus* as a series of "accessory optic vesicles" lying behind the true optic vesicles of the lateral eyes. Hill ('00) has confirmed the presence of these accessory optic vesicles in his study of the chick. Nothing of this kind however seems to appear in *Amblystoma*. The pineal eye forms very late. No new facts could be discovered either in support or in refutation of Locy's theory.

The post-cephalic region at the time the neural crests close over presents a very characteristic appearance due to the presence of narrow shallow grooves running across the plate transversely and obliquely in no regular pattern (fig. 8, A). This is one more factor which aids in marking off the procephalic lobes from the remainder of the plate.

Stage 9 (fig. 12). A few figures of older embryos are introduced here in order to carry the work to a point where the more important landmarks of the adult brain may be readily recognized. Fig. 12, B and C, are side views of embryos just old enough to show the otic pit (op) and the swellings of the brain tube called by Kupffer secondary neuromeres. The changes that take place between the closing of the neural tube and the condition represented by these older embryos are readily understood. Fig. 12, A, is a view of an embryo in which the neural crests have already fused. The optic vesicle is divided by a faint groove or constriction into eye stalk (es) and eye vesicle proper (e). The mid-brain is bilobed as in the preceding stage (mb1, mb2). The cerebellar swelling (m1) now begins to separate a little from the neural crest. A series of new swell-

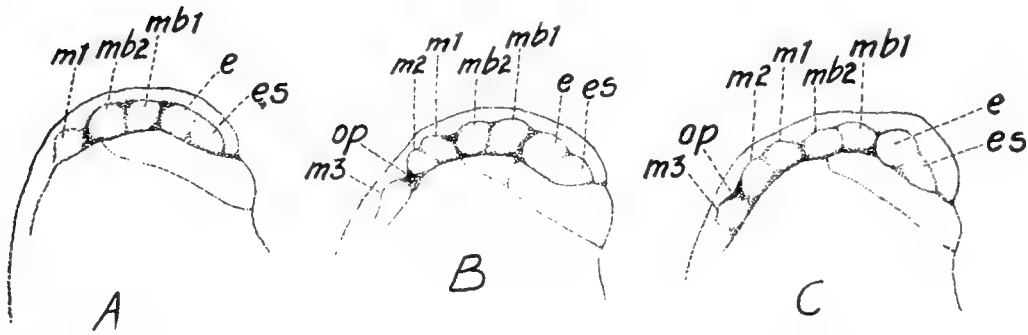


FIG. 12. Side views of the neural tubes of older embryos, stage nine. e, eye; es, eye stalk; m1, m2, m3, neuromeres of the hind-brain; mb1, mb2, divisions of the mid-brain; op, otic pit.

ings now appears in the hind-brain back of the cerebellum forming in order from in front backward, the completion of which is seen in the older embryos B and C. (m1, m2, m3). Between the second and third swelling appears at this time a pigmented crescent shaped depression the otic pit (op). The presence of these various swellings along the neural canal gives a very characteristic segmental or beaded appearance.

This description of the swellings or "neuromeres" of the closed neural tube agrees essentially with the results of other authors. The agreement will be made apparent by a more detailed discussion of the important features.

In the embryonic fore-brain of vertebrates Kupffer ('03) distinguishes three regions or, as he calls them, "secondary neuromeres," the telencephalon, the parencephalon and the synencephalon. Locy ('95) and Hill ('00) found three primary neuromeres in the fore-brain before the development of the secondary divisions but they could not trace the primary divisions into the secondary ones. Neal ('98) considered the fore-brain to be one large neuromere which later showed secondary divisions. The relation of the first procephalic neuromere to the fore-brain has already been discussed. In *Amblystoma* the telencephalon appears as Kupffer has described it but it develops so late that no satisfactory explanation of its relation to the primitive segmented neural axis can be given. Behind the telencephalon lies a division called by Kuffer the parencephalon. The lower



half of it comprises the infundibulum or infundibular depression and the base of the eye stalk. Between the parencephalon and the mid-brain is a small wedge-shaped region, the synencephalon, a region which in fish embryos is prominent but which is hardly noticeable in *Amblystoma*. The temporary appearance in the mid-brain of two swellings is, according to Kupffer, almost universal among vertebrates. It has been found in various types by Locy, Hill and Johnston. It is undoubtedly present for a short time in the optic lobes of *Amblystoma* but it soon disappears and the mid-brain becomes an unsegmented region with a narrow ventricle and thick walls as shown in the older embryo seen in fig. 12, D. In the hind brain the most important swelling has been called the cerebellar crest. As first described it lies directly behind the mid-brain but in older stages it migrates slightly backward leaving a triangular region in which the adult cerebellum arises. This triangular region is called by most authors a distinct cerebellar neuromere. *Amblystoma*, and according to McClure ('90) Kupffer ('05) and Johnston ('05) all the Amphibia as well, lack the so called "blank neuromere" which is found in the embryos of many vertebrates just behind the swelling described above. The second swelling in the hind-brain of *Amblystoma* resembles the first. Behind the otic pit lies a third swelling resembling the first two. These swellings of the hind-brain may well be called true neuromeres since as Johnston and Locy have shown they are like the undoubted segments of the spinal cord. and since there are no accessory structures behind the cerebellum to produce secondary divisions.

There are then in the closed neural tube of *Amblystoma* a series of swellings extending from the anterior end of the brain to the otic pit, but since these divisions are of varying morphological significance they cannot rightly be called neuromeres. If no such general term is applied to them as a group considerable confusion may be avoided, for then the morphology of each may be investigated on its own merits as it has been the aim of this paper to do.

## GENERAL CONCLUSIONS

The foregoing description, whatever may be the value of the results attained, emphasizes the value of breaking away somewhat from the traditional methods of attacking the problem of cephalogenesis. Two new lines of work have been followed.

*First.* The number of neuromeres is not the all important part of the problem. Since the time of Goethe students of cephalogenesis have bent their energy mainly to finding out how many segments the head contained. Even if the search had led to a definite result, which has been far from the case, the main problem is far from being solved. The grouping of neuromeres into tagmata may prove to be more important than the mere number involved which may possibly vary. Johnston's theory of longitudinal zones is a welcome breaking away from the old method of counting neuromeres, and a study of germinal grooves and optic lobes may prove to have still greater value.

*Second.* Very early stages should be used for study. Important structures appear and disappear before the formation of the conventional neuromeres. This point has all along been maintained by Kupffer who has claimed that the only true primary neuromeres must be sought for in the open neural plate. It is evident in spite of all the recent investigation of the closing and closed neural tube that there is yet a broad field for study in the open plate and its allied structures.

The results obtained by these two new methods, so far as the bearing of the minor points of interest is concerned have already been discussed along with the descriptive part of this paper. It now remains to consider the general bearing of these results upon the wider problem of cephalogenesis. It will be convenient to employ the headings used by Locy ('95) in his historical survey.

*First.* "The primitive condition of the nervous system of vertebrates." The evidence presented in this paper supports the view that the nervous system of ancestral vertebrates was not a simple but a complex one. In the history of Amblystoma embryos a glimpse is afforded into conditions and processes which must have been developed before the evolution of modern verte-

brates since they are not seen in any adult vertebrates even in the simplest forms. The nucleus of the nervous system is a segmented solid axis but the complexity of this axis is considerable when we take into consideration the peculiar history of the first neuromere. And again there is evidence of the grouping of neuromeres into tagmata, a process which must have had an ancient origin in the phylogeny of vertebrates. The complexity becomes greatly increased when it is considered that to this neural axis are added accessory structures such as germinal grooves, retinal spots, optic lobes and neural crest, the absorption of which into the neural axis takes place very early in embryonic life. It may readily be admitted that several more accessory structures are really represented although they cannot be detected in *Amblystoma*. At any rate enough has been proved to show that the very early anlage of the nervous system of so simple a vertebrate as *Amblystoma* is comparatively complex.

*Second.* "The number and nature of the primitive neural segments." The exact number of segments in the brain is perhaps of less importance than the grouping of segments into tagmata. It has been shown that the anlage of the fore-brain, the mid-brain and the cerebellar region is a tagma of four neuromeres. It is possible that the exact number of neuromeres may vary in different vertebrates, the tagma themselves forming the invariable units. These early segments are evidently true primitive neuromeres while the later divisions of the neural tube are evidently secondary due to the presence of secondary structures such as the optic lobes.

*Third.* "The line of modification" of neuromeres. It has been shown that the vertebrate brain is not derived solely from a simple series of neuromeres but that its anlage is complex containing structures lying lateral to the segmented neural axis. Thus the process is not merely a "modification of neuromeres" but comprises a fusion of more or less independent anlagen. To the first neuromere after it has been folded down into the infundibular depression are added the eye vesicles, the origin of which is lateral to the neural axis. Possibly also the optic thalami and the parietal eye which appear later in the walls of this region have a

similar origin. The relation of the hemispheres to the first neuromere presents a difficulty to which Amblystoma seems to afford no solution. To the second and third neuromeres are added the optic lobes to form the peculiar sides and roof of the mid-brain, a history which is very clear and definite in Amblystoma forming the most valuable contribution of this paper. At the sides of the closing neural tube in the region of the fourth neuromere develops the cerebellar crest. To the history of the modification of the neuromeres of the medulla no facts were discovered to add to the account already given by Locy and other authors.

*Fourth.* "The differentiation of sense organs." Two facts have been discovered. The exact position of the anlage of the lateral eye has been shown to be just lateral to the neural plate, not on it. Not only is this anlage incorporated into the neural tube but also its ganglion, the optic lobe, is incorporated to form the roof and sides of the mid-brain.

#### SUMMARY

I. Four grooves appear on the embryonic area of the egg in the following order: the posterior germinal depression, the anterior germinal depression, the blastogroove, the neural groove. The last named alone persists until the neural tube is formed.

II. The axis of the nervous system is the neural plate which is divided longitudinally by the neural groove and is surrounded by the peripheral groove.

III. The neural crests arise independently of the neural plate, being separated from it by the peripheral groove.

IV. The anus arises as a posterior prolongation of the blastogroove.

V. The anterior end of the neural plate, the procephalic lobes, is marked off from the rest of the plate by a deep transverse groove and also by its darker color, its greater width, and its elevation above the rest of the plate.

VI. There are four neuromeres in the procephalic lobes.

VII. Neuromeres appear behind the procephalic lobes but owing to the fact that they are irregular and quickly disappear their history cannot be followed.

VIII. The anlage of the lateral eye and the anlage of its ganglion, the optic lobes, are seen lying lateral to the neural axis before the closing of the tube. The optic lobes form the roof and sides of the mid-brain.

IX. The neural crests first close together just in front of the anus, then coalesce slightly at the anterior end, then meet over the procephalic lobes. From these points fusion proceeds in both directions.

X. The anterior neuropore remains open for a very short time.

XI. The anlage of the ear is pigmented.

XII. The division of the closed neural tube into a series of swellings is present in *Amblystoma* but these divisions do not correspond exactly to the true neuromeres of the open plate.

XIII. Since the cerebellar swelling is found at the sides of the fourth neuromere of the procephalic lobes, evidently these lobes occupy a region corresponding to the fore-brain, mid-brain and a portion of the hind-brain.

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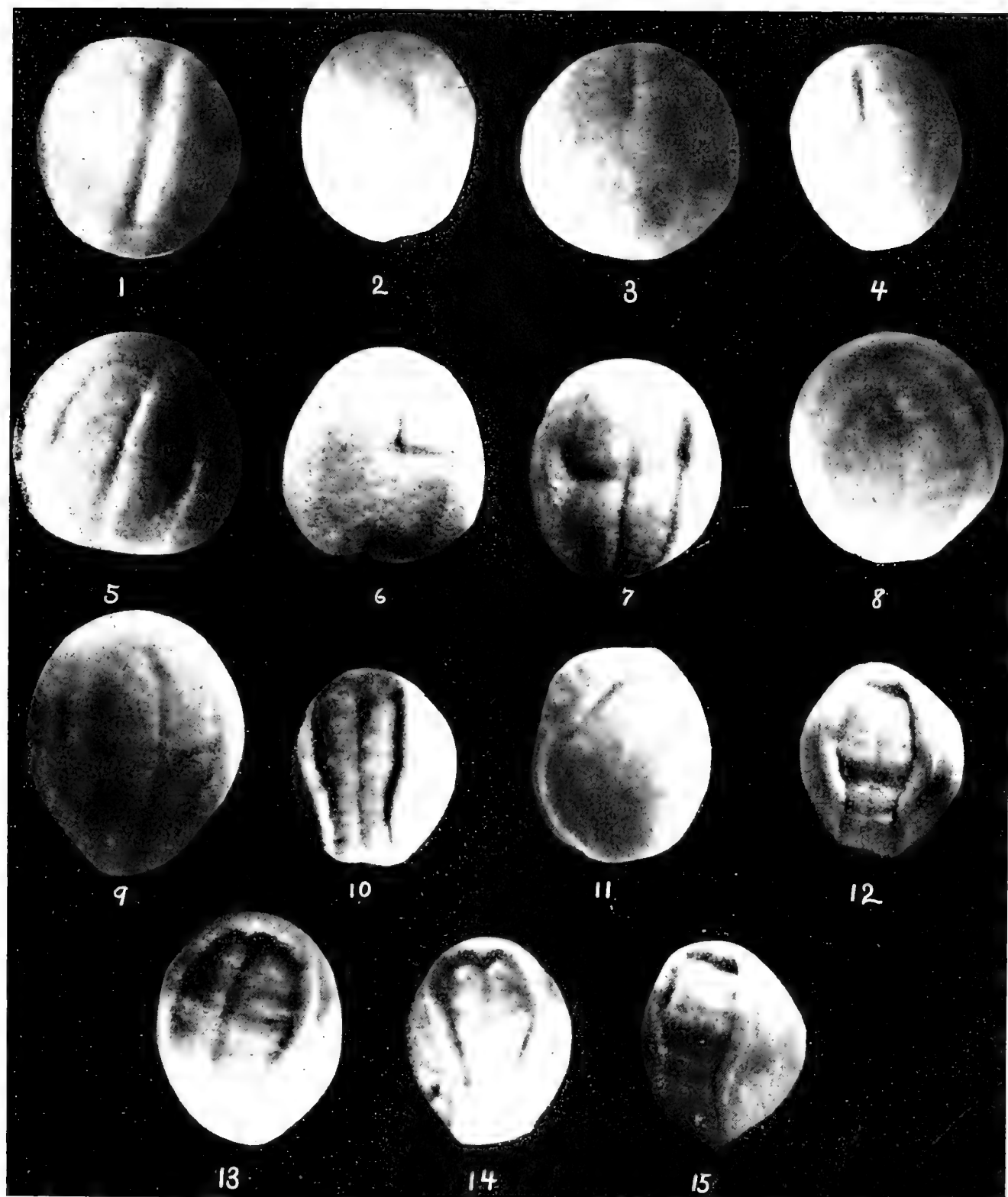
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## PLATE I

### EXPLANATION OF FIGURES

1. Spherical egg showing a short anterior and a long posterior depression.
2. The two germinal depressions very closely united.
3. The anterior germinal depression after the posterior depression has disappeared.
4. The anterior germinal depression, with the posterior depression at the point of disappearing.
5. Appearance of the peripheral groove marking out the area of the neural plate. The anterior and posterior germinal depressions are distinguishable and there is the beginning of the transverse cephalic groove on the left side.
6. The transverse cephalic groove fully formed on the right side. The posterior germinal depression is absent.
7. A very deep transverse cephalic groove particularly on the left side. The newly formed neural groove is seen, being especially well marked in the posterior half of the neural plate. The neural crest is visible on the right side.
8. The faint appearance of one neuromere. The neural crest is fully formed.
9. The appearance of one neuromere at the posterior end of the cephalic plate. This photograph shows the relation of the neuromere to the anterior germinal depression.
10. The appearance of all four neuromeres. The first is the least defined and the second is much the largest.
11. The four neuromeres seen from the side. The posterior borders of the second, third and fourth neuromeres appear as dark bands. The position of the first neuromere is well defined. The procephalic lobes are raised above the level of the rest of the plate.
12. The infundibular depression and behind it three well marked neuromeres. the second, third, fourth.
13. Infundibular depression and three neuromeres.
14. Infundibular depression and three neuromeres.
15. Infundibular depression and three neuromeres.





# THE DEGENERATED CELLS IN THE TESTIS OF LEPTINOTARSA SIGNATICOLLIS<sup>1</sup>

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NINE FIGURES

The present contribution comprises a detailed account of a peculiar process of degeneration or cytolysis of certain cells in the testis of the chrysomelid beetle, *Leptinotarsa signaticollis*, the occurrence of which was noted in a previous publication (Wieman, '10). Since then I have been able to study the process more minutely, with the result that I can now give a more complete account of its history.

The testes are two in number and lie one on either side of the body. Each testis of the imago consists of two disc-shaped lobes bound together by a common sheath of epithelial cells. Two ducts, one from the flattened proximal end of each lobe, unite to form a common duct on either side which in turn unites with its fellow from the other side to form the single median efferent sperm duct (fig. 1). Each lobe, except in the region opposite the point where the duct leaves, *i.e.*, the distal end, is divided into follicles separated from each other by ingrowths of epithelial cells from the investing sheath (the sheath has been omitted from the figures for the sake of simplicity). The follicles, radiate about the central cavity of the testis (which is continuous with the cavity of the duct) much in the manner of the spokes of a wheel about the hub. They contain cysts of germ cells in various stages of development, while the central cavity in the mature organ is filled with spermatozoa.

<sup>1</sup> I take pleasure in acknowledging my obligation to Professor W. L. Tower of the University of Chicago, to whom I am indebted for the material upon which the present study was based.

Externally the distal end of the lobe is marked by a round cap-shaped depression (fig. 1, *d*). A section through this region (fig. 8) shows that the cap is composed of (1) an outer layer made up of two kinds of cells, viz., spermatogonia (*sp.*) that have not been grouped into cysts, and an isolated mass of pale-staining cells

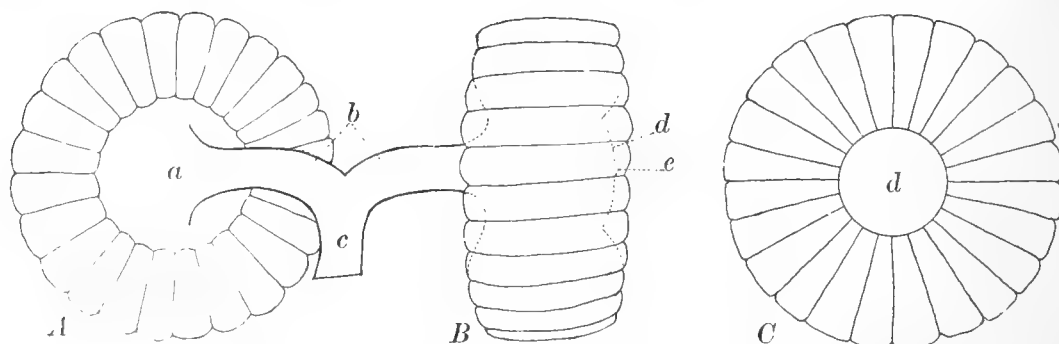


FIG. 1 Diagrammatic drawing of the lobes of the testis. A, proximal view; B, side view; C, distal view; *a*, expanded end of the sperm duct, *b*, at the point where it leaves the testis. This is shown in dotted outlines in B; *c*, common sperm duct of one side. This unites with its fellow from the opposite side to form the median sperm duct; *d*, circular depression opposite the opening of the central cavity of the testis into the sperm duct; *e*, small area or cap of epithelial cells corresponding to the expanded end of the base of the terminal thread of the ovariole.

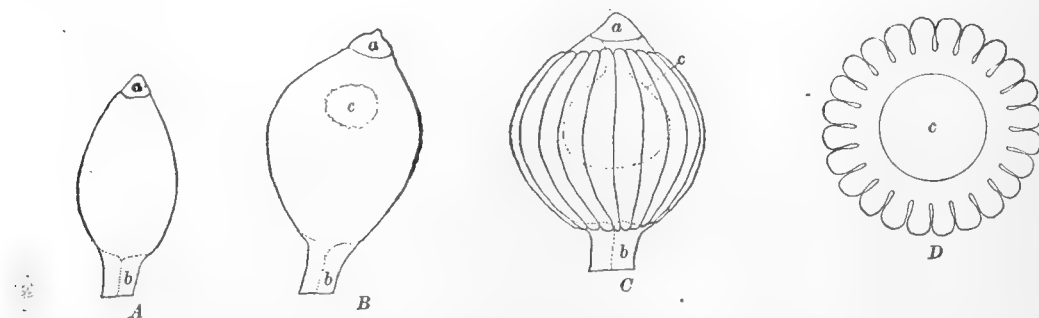


FIG. 2 Diagram illustrating three stages in the development of the testis. A, middle larva period; B, late larva; C, pupa, in which the position of the degenerating cells is indicated by the broken lines; D, transverse section of C in which the degenerating cells fill the central circular area; *a*, terminal cap of epithelial cells; *b*, sperm duct; *c*, degenerating cells.

(*e.c.*); (2) an inner layer of degenerated cell fragments (*d*), which is definitely marked off from the spermatogonia above and the spermatozoa below by a delicate capsule of thin flattened epithelial cells (*e.m.*). It is with the history of the peculiar cytologica

condition, seen in the inner layer, that the present paper has to deal.

In order to facilitate a complete understanding of the facts I shall outline briefly the main features of the development of the testis as a whole. In the earliest stage of larva studied each testis consists of a pair of spindle-shaped lobes (fig. 2, *A*) showing no

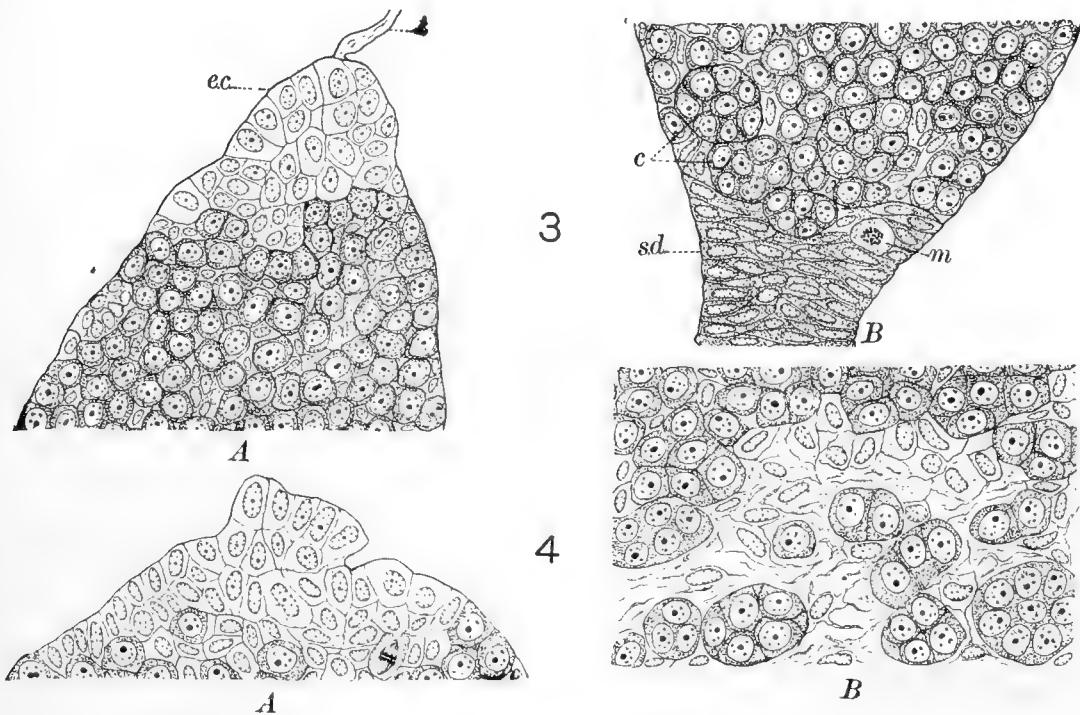


FIG. 3 Longitudinal section of the distal end *A*, and the proximal end *B* of a lobe of a larval testis. *e.c.*, terminal cap of epithelial cells; *s.d.*, sperm duct; *c*, cysts of germ cells; *m*, mitotic division in a cell of the sperm duct; *t*, thread-like rudiment comparable to the terminal thread of the ovariole.  $\times 265$ .

FIG. 4 Longitudinal section of a larval testis about two or three days older than that shown in the preceding figure. *A*, distal end; *B*, region slightly proximal to *A*, showing the accumulation of epithelial cells that marks the first step in the degeneration process.  $\times 265$ .

trace of follicular arrangement, and terminating distally in a cap (a) of lightly staining epithelial cells. The first step in the further development of the organ consists in an enlargement in a transverse direction without a corresponding increase in length (fig. 2, *B*). In the next stage (fig. 2, *C*) the follicles appear as radial outpocketings from the sides of the lobes. Fig. 2, *D*, shows

the appearance of such a lobe in cross section. As the follicles grow out their epithelial covering remains closely attached to the outer surface, so that in the fully developed organ the follicles are separated from each other by a layer of epithelial cells.

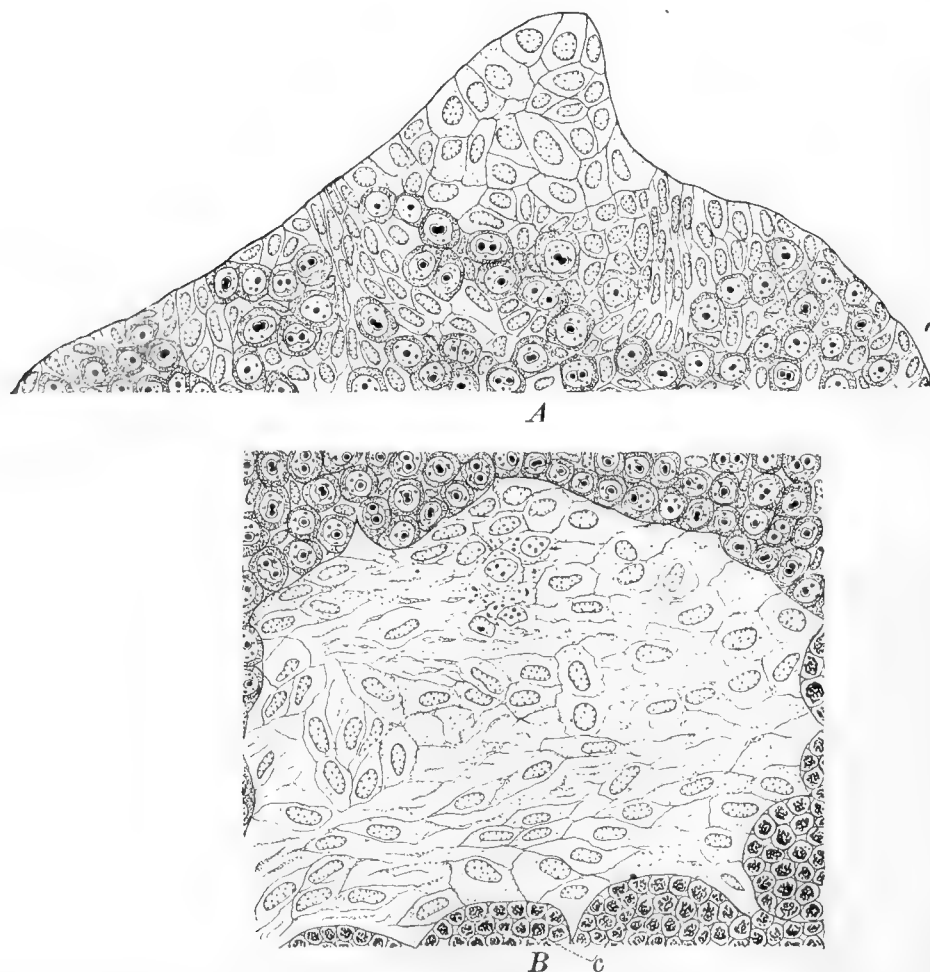


FIG. 5 From larva about two days older than the preceding. *A*, section of the distal end of the lobe; *B*, the degenerating cells; *c*, cysts of spermatocytes.  $\times 265$ .

In the early larval stages each lobe is filled with intermingled germ and epithelial cells as is shown in fig. 3, *A*, *B*. The terminal cap of epithelial cells (*e.c.*) is comparable in appearance and position to the expanded base of the terminal thread of the ovariole of the mature female. A delicate thread-like process is seen at *t* which reminds one of the terminal thread of the ovariole. This appendage does not appear in later stages. The region of the epithelial cap is continuous with the rest of the testis, which, to

make the comparison complete, corresponds to the terminal chamber of the ovariole.

It will be noted that some of the germ cells at the lower (proximal) end of the lobe (*B*) are grouped into cysts (*c*) while elsewhere the cells are free. As has already been pointed out (Wieman, '10), a careful study of these stages led me to believe that each cyst arose from a single mother cell by an amitotic method of cell division, similar to the manner in which cyst formation takes place in the nurse cells of the ovary.

In figs. 3, 5, 8, etc., abundant evidence of amitosis can be seen. The division begins with the constriction of a large basic staining nucleolus into two parts, after which the nucleus undergoes a similar division. Division of the cytoplasm does not take place immediately and as a result bi-nucleated cells are frequently seen. Actual division of the cytoplasm is very difficult to demonstrate, but that it must occur is evident from the fact that the spermatocytes that result from these cells are cells with single nuclei.

After the cysts are definitely formed amitotic figures disappear and all subsequent divisions are mitotic. As cyst formation begins in the lower (proximal) end of the lobe, amitosis disappears first in this region; while in the other parts it continues as long as the spermatogonia are not grouped into cysts. As a result, in the fully developed organ, in the region of the cap in the distal end of the testis amitotic figures abound.

In the larval stage shown in fig. 3 there is no evidence of degeneration. The first indication of such a process is shown in fig. 4, *B*, which is from a larva about two days older, and consists in an accumulation of epithelial cells at a point removed from the distal end of the lobe a distance of one-third the length of the entire lobe. This stage is shown diagrammatically in fig. 2, *B*. The cells are irregular and ragged in outline, and this, together with the fact that there is a considerable amount of inter-cellular substance, suggests a process of liquefaction or secretion. The germ cells are readily distinguished by their large rounded nuclei and deeply staining cytoplasm so that there can be no doubt about which cells take part in the process.



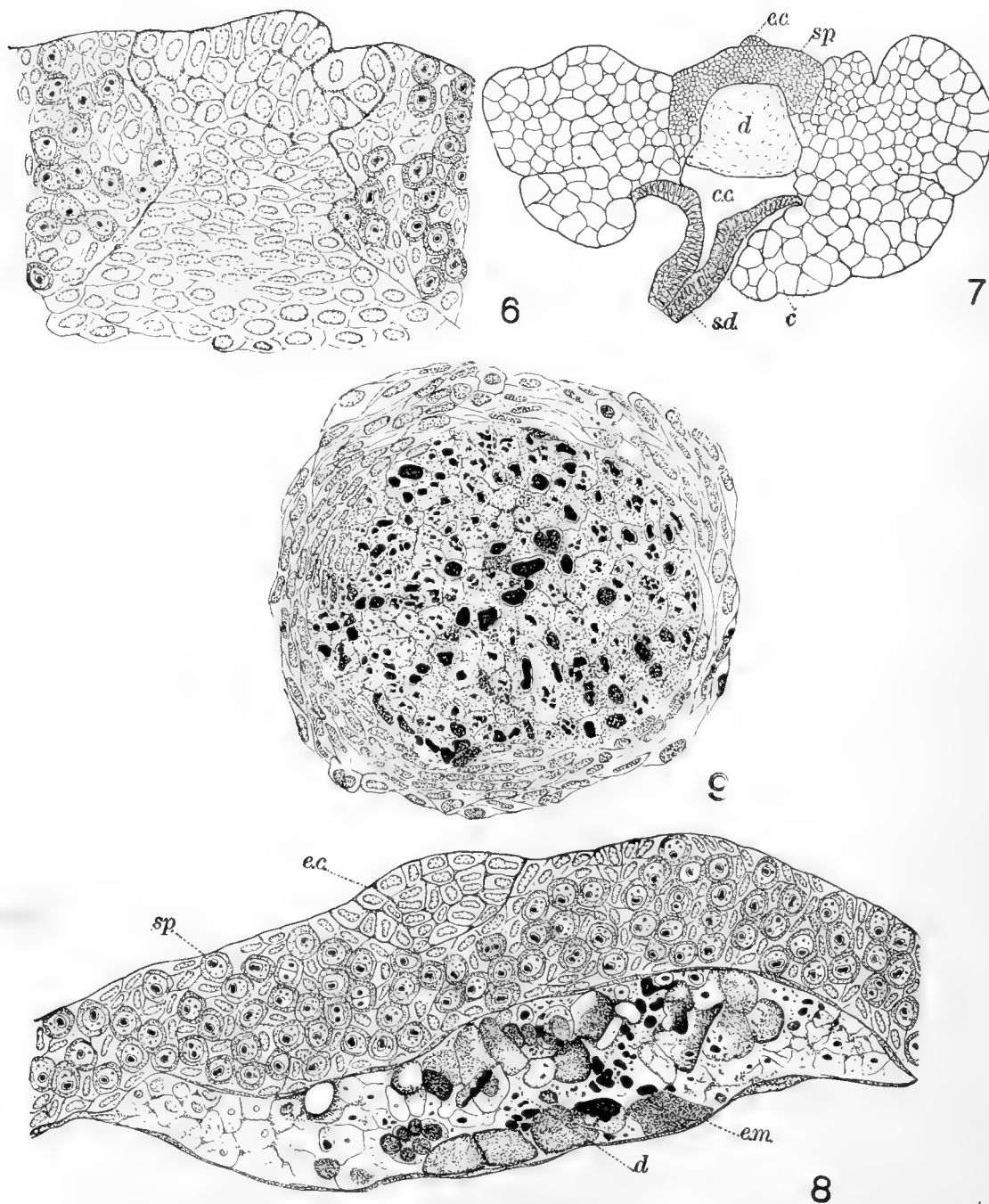


FIG. 6 Section through the distal end of the pupal testis showing continuity between the cells of the terminal cap and those of the degenerating region.  $\times 265$ .

FIG. 7 Complete transverse section of a pupal testis. *e.c.*, terminal cap; *s.d.*, sperm duct; *c.*, cysts of germ cells; *d*, degenerating cells; *c.c.*, central cavity of the testis continuous with that of the sperm duct; *sp*, spermatogonia not grouped into cysts.

FIG. 8 Longitudinal section of the distal region of the testis of an imago. *e.c.*, terminal cap of epithelial cells; *sp*, spermatogonia; *e.m.*, membrane of flattened epithelial cells enclosing the degenerated area. *d*, degenerated cells.  $\times 265$ .

FIG. 9 Transverse section through the distal region of the testis of an imago.  $\times 255$ .

Fig. 5, *A*, *B*, is from a larva approximately two days older than the preceding. The separate follicles have begun to form and the terminal epithelial cap is in the shape of a papillary projection from the flattened distal surface of the lobe. The accumulation of epithelial cells has grown in amount. (With the exception of figs. 1, 2 and 7, all figures are drawn at the same magnification). In the distal (upper) part of this region, deeply staining granules within and without the epithelial cells mark the beginning of the second stage in the degeneration process.

In the case of the ovariole the conclusion was reached that the epithelial cells of the terminal chamber are derived from the pale-staining cells of the terminal thread. Somewhat similarly, in the testis, the cells of the degenerated area have their origin in the terminal cap, which structure is apparently homologous with the enlarged base of the terminal thread of the ovariole. Fig. 2 shows direct continuity between the cells of the degenerated area and those of the terminal cap. Such a condition persists throughout the greater part of the pupal period; that is, until the central accumulation of epithelial cells reaches its maximum size. Toward the end of this period of development the two regions become separated by a layer of spermatogonia, as shown in fig. 7 which represents a section of an entire pupal testis.

Examination of the central cells (*d*) of this figure under higher magnification shows that disintegration has progressed in the distal part, although the cell outlines still persist. The principal change is the large increase in number of cells composing the mass, which is spheroidal in form and approximately fills the central cavity of the testis.

With the disappearance of the connection between the cells of the terminal cap and those of the central region, degeneration and cytolysis among the latter proceeds very rapidly. As the process goes on the mass of cells as a whole shrinks toward the distal end of the central cavity, until in the fully developed testis of the imago it has the appearance represented in fig. 8. Fig. 9 is a section taken at right angles to the axis of the testis and

gives another view of the degenerated area. From these it can be seen that throughout the greater part of this region all trace of cellular structure has disappeared, the cells having been replaced by clumps of material of various sizes, deeply stained by either iron-alum-haematoxylin or basic anilin dyes. The mass is bounded by a membrane of flattened epithelial cells. The terminal cap of cells (fig. 8, *e.c.*) has become reduced in size; it no longer protrudes from the surface and the outlines of the cells are distinctly marked by the presence of a deeply staining substance in their periphery, which together with their shrivelled appearance indicates that these cells likewise are undergoing atrophy.

In the paper referred to before the deeply staining particles of the degenerated region were looked upon as representing the more solid parts of the degenerated cells—it being assumed that the more fluid parts had been expressed into the cavity of the testis as a result of accompaniment of the process of contraction. It can be readily understood that as the degenerated mass is compressed toward the distal side of the testis, the central cavity of the latter comes into existence. The cavity however almost immediately becomes filled with mature spermatozoa, set free by the rupture of the surrounding follicles. These facts called forth the suggestion that the more fluid products of the degenerated cells serve as a nutritive substance, or perhaps a liquid medium, for the spermatozoa.

It is obvious that this peculiar cytological condition is different in every way from the degenerated cells that have been described in the testis of spiders. According to Wallace, in *Agalena naevia* degenerated cells or cell fragments are found enveloping the spermatozoa in the sperm ducts, and are said to come from at least four different sources: (1) broken down walls of empty cysts, (2) cell bodies from which ripe spermatozoa have escaped, (3) "connecting bodies" of sister spermatids and their contained mid-bodies, (4) large oval cells which resemble the rolled up spermatozoa in size and outline.

In *Leptinotarsa*, so far as I have been able to determine, only epithelial cells derived from the terminal cap participate in the

degeneration. Further, the cell fragments are not found mingled with the spermatozoa in the sperm ducts. While I have noted occasionally cysts of degenerated spermatocytes, the fact has no direct bearing upon the matter under consideration.

All of the literature on the histology of the Coleoptera has not been accessible to me, but in that which has been at my disposal, I have not met with any description or reference to the degeneration process as I have observed it in the species under discussion. It is almost unnecessary to remark that the appearance of degeneration is not due to an artefact, the result of the action of fixing agents. The degenerated mass can be detected in all the testes that I have examined in at least two species of *Leptinotarsa* (*signaticollis* and *decemlineata*) however killed and stained, and moreover is most distinct and clear-cut in the best preparations as judged by the fixation of the other parts of the organ.

The cap of epithelial cells from which the degenerated cells arise undoubtedly corresponds to what Demokidoff has described in *Tenebrio*: "Jeder von den 6 Follikeln des Hodens von *Tenebrio molitor* besitzt ein eigenthümliches Gebilde (eine Linse) welches aller Wahrscheinlichkeit nach ein der Endkammer der Ovariolen entsprechendes Organ darstellt." But as I have already pointed out, the structure in *Leptinotarsa* appears to be homologous not with the Endkammer, but with the enlarged base of the terminal thread.

Similar structures have been described in other species of insects, viz., the "Apicalzelle" of the testes of *Diptera* and *Lepidoptera*, which is located in a similar position in the end of the organ. Grünberg speaks of the "Apicalzelle" as the nurse cell of the testis, which when its function is completed degenerates along with a few of the germ cells.

In *Leptinotarsa*, no cells corresponding to the nurse cells of the ovariole are present. The walls of the cysts are formed of epithelial cells similar to those that degenerate, and as the spermatozoa mature a number of them become attached to one of these cells. This "nurse cell" is a greatly enlarged epithelial cell, and not a transformed germ cell as is the case in the ovariole. The

fact that the germ cells are provided with a source of nutrition as long as they remain in the cysts, suggests that if the degenerative process is for the purpose of producing a nutritive substance for the germ cells, this substance is used only after they have been discharged from cysts as mature spermatozoa into the central cavity of the testis and the sperm ducts.

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# CHARACTERISTICS OF THE DIVERSE RACES OF PARAMECIUM

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TWENTY-FOUR FIGURES

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## INTRODUCTION

In an earlier paper (Jennings, '08) the senior author showed that there exist in *Paramecium* (*aurelia* or *caudatum*) many races, differing in size. On account of the extreme importance of this fact in all sorts of systematic and experimental work with such animals, and particularly in work on heredity and variation, it seems well to give an account of the structural and physiological characteristics of these races. Some of the races have now been under observation in the laboratory for more than three years, so that there has been opportunity for a careful study.

In the paper of 1908, Jennings distinguished eight different races, permanently differentiated in mean size. Each race contained individuals of varying size, owing to growth and environmental influences, but under identical conditions each race showed a characteristic average size, differing from that of the others.

Do these races differ in other respects as well as in size? In the paper of 1908 the only other differences set forth were the following: (1) the smaller races are on the average a little thicker in proportion to the length than the larger (pp. 500-503); (2) one of the larger races, *D*, had a single micro-nucleus; one of the smaller, *c*, had two (p. 500). The relation between these races and the supposed distinction between *Paramecium aurelia* and *P. caudatum* was briefly discussed, without reaching a definite conclusion.

The present paper is divided into two parts. Part 1, by Geo. T. Hargitt, deals with the number and structure of the micro-nuclei and the relation of the races to the two supposed species, *Paramecium aurelia* and *P. caudatum*. Part 2, by H. S. Jennings, takes up (1) the permanence of the differences in size; (2) the similarities and differences among the various races in the form of the body and in other structural features; (3) differences with respect to conjugation; (4) differences in rate of fission; (5) differences in cultural requirements, and other physiological differences; (6) the relation of these races to the various described species of *Paramecium*; (7) the occurrence of such races in other Protozoa, and (8) in higher organisms; (9) certain theories of the origin of these races; (10) a list of the various races and lines studied,

with brief characterization; (11) precautions necessary in working with pure races.

PART 1. NUMBER AND STRUCTURE OF THE MICRO-NUCLEI,  
AND THE SPECIES QUESTION

GEO. T. HARGITT

The organisms in which we are interested were first described by Müller in 1773 as *Paramecium aurelia*. A fuller account, illustrated by figures, is given in Müller's work of 1786. Müller's account fits any or all of the different races. Ehrenberg ('38) attempted to distinguish two forms, a larger one, pointed behind, being given the name *Paramecium caudatum*, while Müller's name *aurelia* was reserved for a smaller form, having the posterior end less drawn to a point. There has been much difference of opinion and discussion among later authors as to whether this distinction was justified, and the two names *aurelia* and *caudatum* have been used by some authors in a more or less indiscriminate way. The history of the matter down to the time of Maupas cannot be summarized better than in Maupas' own words: "Ehrenberg and Dujardin had distinguished two large Paramecia, *P. caudatum* and *P. aurelia*. Their distinction was based solely on external characters, the first species being held to be more fusiform and elongated, the second broader and stouter. Stein and Claparède, still from the study of external features, contested the validity of this distinction and reunited the two species under the name of *P. aurelia*, thus suppressing *P. caudatum*. This reform was accepted by all the observers that followed. It is thus that Balbiani, Stein, Koelliker, Engelmann, Bütschli and Gruber, who have studied the conjugation of a large Paramecium, have all given it the name of *P. aurelia*. It is further beyond question that they have all dealt with one and the same species. Only Jickeli among recent authors appears to have made anew the distinction of the two Ehrenbergian forms, but he does not tell us on what character he bases it. Nevertheless it is very real, as I hope to demonstrate." (Maupas, '88, p. 230-231).



It was in 1883 that Maupas first set forth the distinctness of the two species, based on the difference in the number and structure of the micro-nuclei in the two, as well as on more external diversities. He says: "All authors hitherto have described *Paramecium* as possessing only one nucleolus of rather large size, measuring 0.005 to 0.008 mm. This is indeed the form most frequently met. But I have observed also numerous individuals provided with two nucleoli, smaller and of different structure from the preceding. They have a spherical form and are composed of a central opaque corpuscle, staining deeply with dyes and measuring only 0.003 mm., enveloped by a cortical layer 0.005 mm. in diameter, transparent and not staining." (Maupas, '83, p. 660). In his paper of 1888 he repeats this description of the difference between the nucleoli, or micro-nuclei as he now calls them. He adds that the number and structure of the micro-nuclei are the distinctive and most important characters of the two species. He did call attention to certain other differences; *P. aurelia* being smaller with the posterior end rounded and having the two micro-nuclei, while *P. caudatum* was a larger form with a single micro-nucleus and a pointed posterior end. The first form, he says, appeared always and only as *P. aurelia*, the latter always and only as *P. caudatum*; the differences, both anatomical and physiological, being sufficient to justify the distinction into two species.

R. Hertwig ('89), in agreement with this distinction, used *P. aurelia* for his study of the conjugation. He says, regarding the micro-nuclei, that there are constantly two in this species but only one in all other species. The two micro-nuclei are usually close to each other and to the meganucleus, though commonly not enclosed within a niche of the latter as was true in other species. Each micro-nucleus, enclosed within a membrane, had a rather densely staining nucleolus composed mainly of chromatin, and a cortical part not staining. This is of course quite confirmatory of Maupas' description of the structure of the micro-nuclei in the same species. Both Maupas and Hertwig found the micro-nuclei to behave somewhat differently from those of *P. caudatum* in the conjugation process.

The work of these two investigators thus appeared to establish

the distinctness of the two species, and it has been generally acknowledged since then. This distinction was accepted by Bütschli in his great work on the Protozoa ('89);<sup>1</sup> and by Schewiakoff ('96) in his monographic treatment of the holotrichous infusoria. Simpson in 1901 says "There is no doubt, however, that these two species do exist." In 1906 Calkins brought the matter into question again upon the following grounds: From a "wild" culture brought into his laboratory in March, 1905, he isolated four pairs of conjugating "caudatum" forms. Upon examining the progeny of one of the exconjugants, after the third division, to determine whether the conjugation had been normal, he found that this exconjugant had "reorganized as an aurelia form with two micro-nuclei." The later behavior of this line he describes as follows: "During the month of May and until three months after the culture was started, individuals appeared here and there with but one micro-nucleus, while the majority of these killed at this time appeared with one of the micro-nuclei larger than the other. By the end of June none of the *P. aurelia* forms were to be found, and this culture, like the other cultures started at the same time, contained forms with only one micro-nucleus; *Paramecium aurelia* had become *Paramecium caudatum* again." (Calkins, '06, p. 5.) He concludes from this that *P. aurelia* is only a variant of *P. caudatum* and states further that it is relatively rare in nature.

The question as to what names we should give these organisms is of course of very subordinate interest; what is of interest is to know the facts as to the permanent differentiations of the various lines or races that exist, whether we call them species or not. I have, therefore, made a careful study of the micro-nuclear relations of the various races of *Paramecium*, to see whether there exist any permanent differences in these structures.

The diverse races or "pure lines" of *Paramecia* studied are mainly those described in the paper of Jennings ('08; see especially pp. 485-500). Each of these races consists of individuals derived from the fission of a single specimen which had been isolated from

<sup>1</sup> It may be noted that Kent in his *Manual of the Infusoria* ('82) recognizes but one of these species, under the name *P. aurelia*.

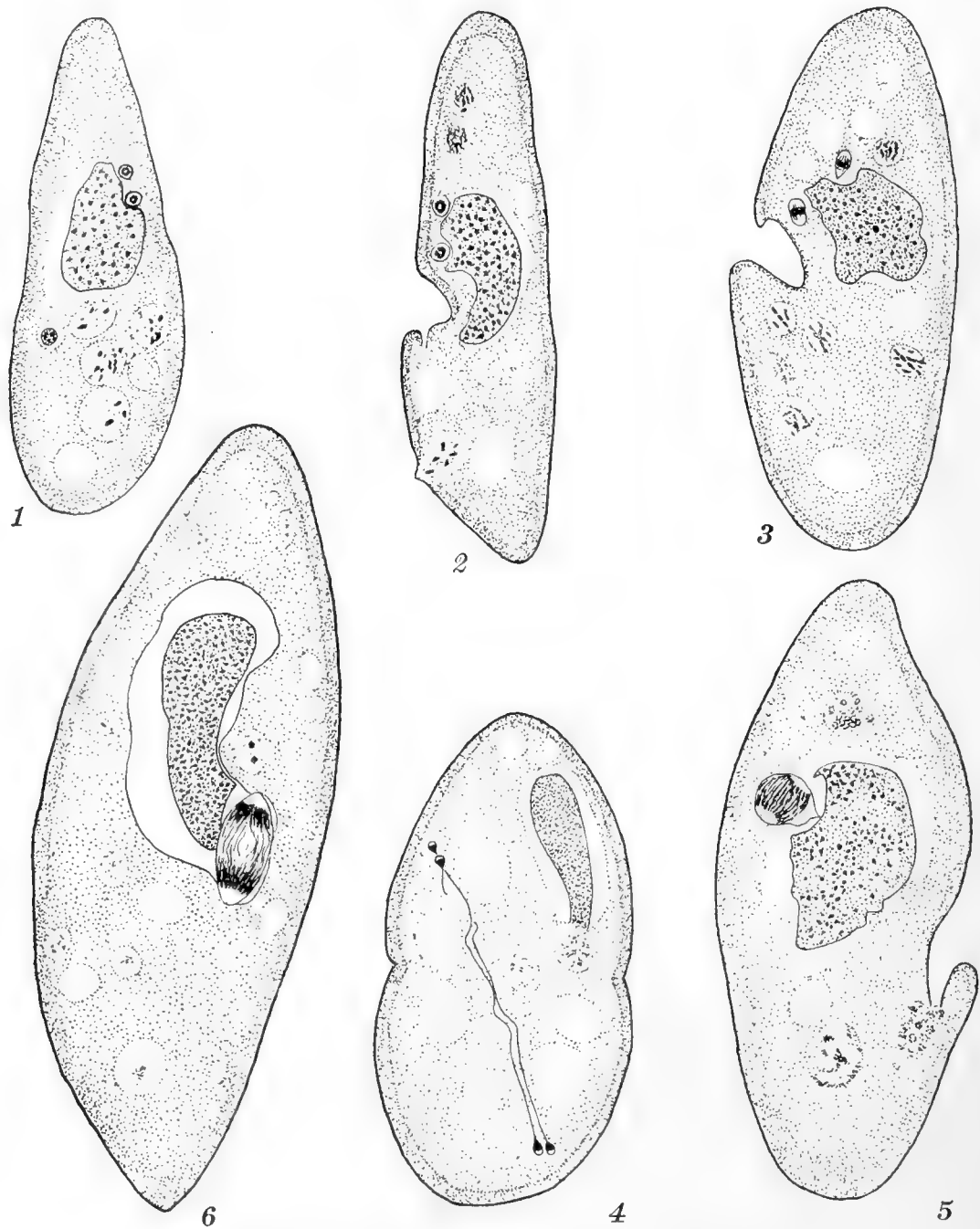
a "wild" culture. These races, as we shall see later, have bred true to their diverse sizes for periods up to three years. The micro-nuclear relations were determined quite independently of any knowledge of the relative sizes or other distinguishing features of the races, in the following way: The samples given me from which to start my cultures were designated by letters only. I received seven of these samples, labeled respectively  $C_2$ ,  $L_2$ ,  $D$ ,  $ko$ ,  $kha_1a_2$ ,  $c$ ,  $i$ . I was quite unfamiliar with the history and characteristics of these various lines, and only after the number of micro-nuclei was determined for each was reference had to the literature on the subject or comparisons instituted with regard to size and number of nuclei and the like. Thus the facts were first determined quite independently of any possible interpretations.

The animals are too thick to allow of the easy observation of micro-nuclei upon whole specimens even when stained and cleared. Therefore it was decided that sections should be used. At the first several killing fluids were tried, picric acid mixtures, mercuric chloride mixtures, formalin, osmic acid mixtures, etc.; also various staining agents were tried with each killing fluid. No essential difference was observed in the various methods and a single plan was therefore followed throughout. For killing, Worcester's fluid (10 per cent formalin saturated with mercuric chloride) was used hot for a few minutes, and the sections stained with Heidenhain's iron-hematoxylin, or with Ehrlich's hematoxylin and eosin. The procedure was identical in all cases. By killing the animals just before they divided, the micro-nuclei were usually found to have moved away from the meganucleus and to have begun their preparation for the ensuing division, or perhaps to be in the act of dividing. This made it possible to determine whether division was progressing normally, and if two micro-nuclei were present to determine whether and how both were dividing. Also it gave a possible chance of finding how the assumption of two micro-nuclei in a race with only one as a rule came about, if indeed such a thing did occur. For each race, therefore, specimens dividing or about to divide were used; for comparison specimens from each race not in the dividing condition were sectioned.

In the determination of the number of micro-nuclei about ten to twenty individuals upon each slide were examined, section by section. The ones picked out for this careful study were mainly those in which the plane of the section was nearly parallel with the long axis of the body; specimens which appeared to be most normal in shape and in the appearance of the cytoplasm, etc., were the only ones included. That is, if specimens were in an abnormal state as regards cytoplasm or meganucleus, they were not considered. The animals were killed and sectioned at different periods, sometimes several weeks or a month apart, and again within a few days of each other. The small number carefully examined upon each slide and the large number of slides thus treated would seem to give a very fair average of the conditions of the entire culture for the entire period of the investigation (four months). While no exact tally of the individuals carefully examined was kept, it is certain that one hundred or more for each culture is below rather than above the probability. Besides, several times as many from each culture were gone over less carefully with confirmatory results.

In brief the results obtained are these: in the races labeled *D* and *L*<sub>2</sub> only a single micro-nucleus was present. In the strains *c*, *i*, *ko*, *kha*<sub>1</sub>*a*<sub>2</sub>, *C*<sub>2</sub> there were two micro-nuclei in each. The structure and staining reaction of the single micro-nucleus was like that commonly described and figured. In the races with two micro-nuclei these agreed in almost every detail with the descriptions given by Maupas and Hertwig and summarized earlier in this paper. Figs. 1 to 11 with their accompanying explanations will serve to show the characteristic appearance and position of these two sorts of micro-nuclei. In addition to the size and structure of the micro-nuclei the external characters of the races agreed in general with the descriptions given by earlier writers for the "aurelia" and "candatum" forms.

If now the races be arranged in the order of their size as found by Jennings ('08), we have (the largest first); *L*<sub>2</sub>, *D*<sub>2</sub>, *k*, *C*<sub>2</sub>, *c*, *i*, and of these *L*<sub>2</sub> and *D* had each a single micro-nucleus, while the other races each had two micro-nuclei (I now found that *ko*, and *kha*<sub>1</sub>*a*<sub>2</sub> were of the same race), *i.e.*, the larger forms had a single micro-nucleus while the smaller ones had two micro-nuclei. A com-



Figures 1-11 are from sections, and are drawn with the aid of the Abbe camera lucida.

Fig. 1. Individual of race *i*. Micro-nucleus in resting condition.  $\times 536$ .

Fig. 2. Individual of race *k*. Micro-nuclei in resting condition.  $\times 536$ .

Fig. 3. Individual of race *c*. The micro-nuclei are preparing for division.  $\times 536$ .

Fig. 4. Individual of race *C*<sub>2</sub>. The micro-nuclei have completed their division and the cell body is beginning to divide. A portion of one micro-nucleus and the meganucleus are added from a second section.  $\times 536$ .

Fig. 5. Individual of race *D*. The micro-nucleus is preparing for division.  $\times 536$ .

Fig. 6. Individual of race *L*<sub>2</sub>. The micro-nucleus is undergoing division.  $\times 536$ .

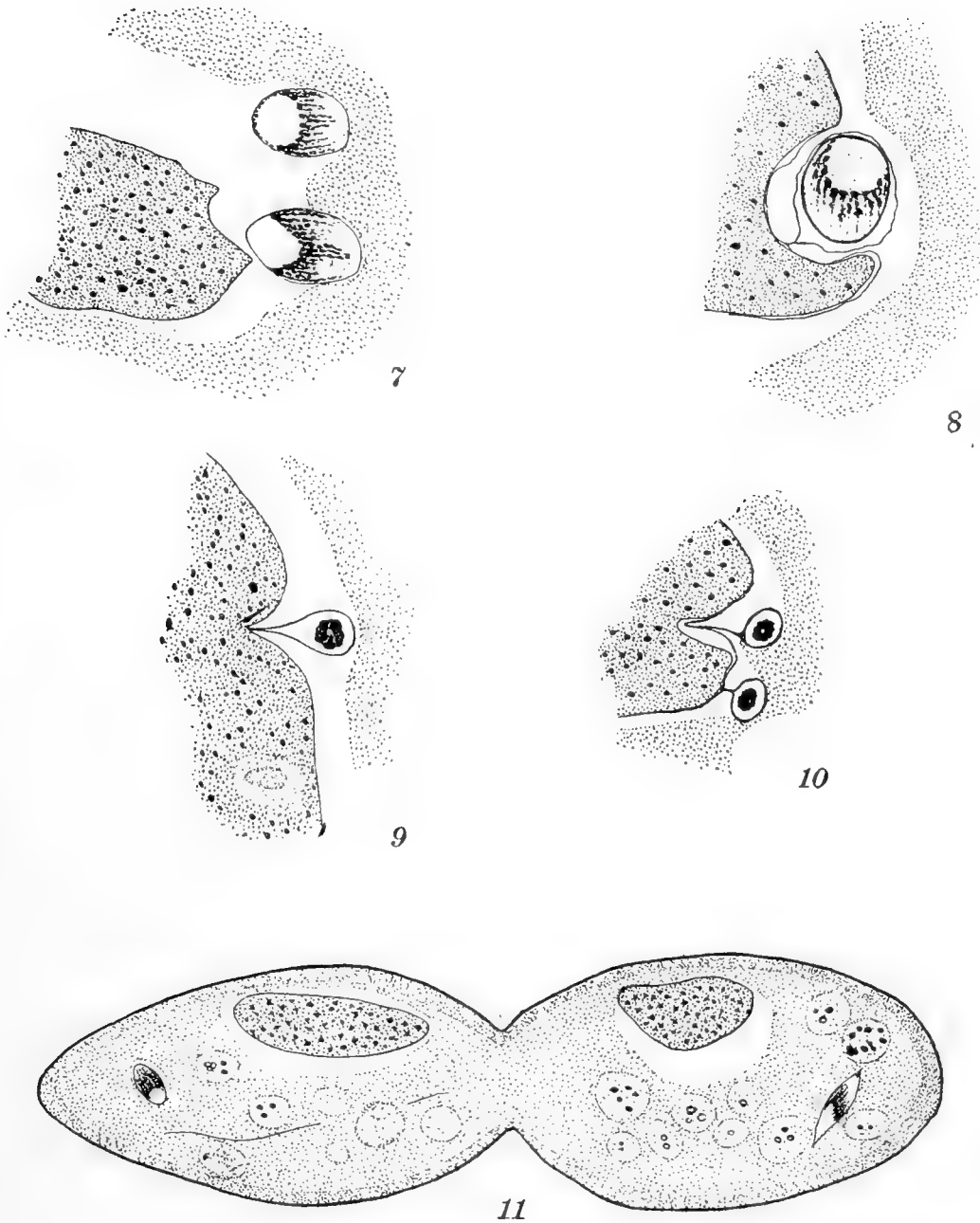


Fig. 7. Micro-nuclei in the resting condition, and a portion of the meganucleus of an aberrant individual of the "caudatum" race  $L_2$ .  $\times 1425$ .

Fig. 8. Resting micro-nucleus and a portion of the meganucleus of a normal individual of the "caudatum" race  $L_2$ .  $\times 1425$ .

Fig. 9. Micro-nucleus in resting condition, and a portion of the meganucleus of an abnormal "aurelia" form, race  $i$ .  $\times 1425$ .

Fig. 10. Resting micro-nuclei and a portion of the meganucleus of a normal individual of an "aurelia" race  $k$ .  $\times 1425$ .

Fig. 11. Dividing individual of the race  $L_2$ . In the anterior half (right in the figure) the micro-nucleus appears as if preparing for a second division.  $\times 536$ .

parison with the earlier suggestions of Jennings is of interest in this connection. He says (p. 500): "I believe it will be found that most *Paramecia* can be placed in one of the two groups that we have called 'caudatum' and 'aurelia' . . . most lines will have a mean length either below 145 microns or above 170 microns." "Furthermore, I am inclined to believe that those belonging to the smaller group (mean length below 145 microns) will be found to have as a rule two micro-nuclei; those belonging to the large group but one micro-nucleus." The prophecy is thus most strikingly confirmed. It is important to remember that these results were not obtained from a single examination of a few individuals at one time. They represent the results of the examination of from a hundred to several hundred random individuals taken from the cultures at intervals of a few days in some cases, and in other instances at intervals of a month or two. Further, since no attempt was made to have the cultural conditions the same, it can not be claimed that the results are dependent upon the constancy of their environment. While hay infusion was used for the culture medium in each case, the hay came from different sources, was sometimes boiled and sometimes fresh. The amount of water and of hay used, and consequently the strength of the infusion, varied considerably. This, it is believed, makes a nearer approach to the natural conditions than would result from the maintenance of constant and uniform conditions in the culture media. But in spite of the conditions, which were different at different times, the number of micro-nuclei was constant. The number of micro-nuclei had been determined three years earlier by Jennings ('08, p. 500) for two of these races, *D* and *c*, and found to be the same as at present.

Constant though these races on the whole were in the number of micro-nuclei, there were a few individuals which varied. Thus, the races which have the single micro-nucleus show occasional individuals with two micro-nuclei; the smaller forms less often have a single micro-nucleus instead of the normal number, two. The number of variations is very small, however. Thus there were found in all the races only eighteen variants, which out of about a thousand individuals carefully examined gives a ratio of only about



2 per cent variation. In the search for these variations every individual which appeared unusually large or small, or in which there appeared to be any indication of more—or less—than the usual number of micro-nuclei, was very closely examined. The chances are that an exhaustive study of every specimen upon every slide would decrease rather than increase this ratio.

The occurrence of even a few variations in the micro-nuclei within pure races is of course in agreement with the facts set forth by Calkins, and shows that there is some basis for his contention that one form transforms into the other. This matter, however, takes on a very different aspect as soon as the *structure* of the micro-nuclei is taken into consideration. Maupas and Hertwig both described a characteristic structure of the micro-nuclei of the aurelia form that was different from that of the caudatum form. The drawings which represent the different races already described (figs. 1–10) show that this difference of structure is very marked. Comparison of fig. 8 (caudatum form) and figs. 9 or 10 (aurelia form) brings out the difference in the resting micro-nuclei of the two forms most clearly. The central dark mass, surrounded by a clear “cortical” area, is most characteristic for the aurelia races; actual comparative examination of preparations from the different races impresses one at once with the sharpness of the distinction. Now it was found that in every instance where an individual of a large race had two micro-nuclei these were of approximately the same size and of exactly the same structure as the normal single micro-nucleus characteristic of the race. There was not a single case where either micro-nucleus of a large race resembled the micro-nuclei characteristic of the “aurelia” forms. Fig. 8 shows the normal single micro-nucleus from a race of large individuals (“caudatum” forms), and in fig. 7 are shown the two micro-nuclei as they occur in each of the aberrant forms of the same race. And the converse is also true. Fig. 10 represents the structure and arrangement of the normal two micro-nuclei characteristic of the “aurelia” forms. When a single micro-nucleus was occasionally found in an individual of these smaller races (fig. 9) it was in every respect like the normal nuclei of the “aurelia” forms and never resembled in any way the micro-nucleus of the “cauda-



tum" forms. From these facts but one conclusion can be drawn, viz., that the two groups not only show a relatively great constancy in the number of micro-nuclei, but the micro-nuclei always differ in structure whether their number is aberrant or normal. Hence the "caudatum" forms are demonstrably distinct from the "aurelia" forms even when they have the same number of micro-nuclei. On this basis the probable explanation of Calkins' case is this: An aberrant "caudatum" form with two micro-nuclei was the starting point of his line; at a later period the individuals in this line returned to the normal condition. That is, Calkins was dealing with *P. caudatum* only. If this interpretation is correct his conclusion should have been: *P. caudatum* (with two micro-nuclei) had become *P. caudatum* again (with one micro-nucleus).

This interpretation is borne out by several facts:

1. It is somewhat remarkable that Calkins in his discussion of this matter, a discussion that includes a list of the differences that Maupas has set forth between the two species ('06, p. 2), does not so much as mention the difference in the structure of the micro-nuclei, which had been emphasized by Maupas and confirmed by R. Hertwig;

2. Thus Calkins does not assert that an animal with micro-nuclei having the caudatum structure had changed into one with micro-nuclei having the aurelia structure.

3. All the figures of micro-nuclei published by Calkins in his recent treatise ('09) and by Calkins and Cull ('07) show the typical caudatum structure.

4. The size given by Calkins for the animals in which he noticed the change of number of micro-nuclei ('06, p. 6) is throughout that which in our experience is characteristic of caudatum (average size from 178 to 224 microns). No aurelia race studied in this laboratory has ever approached in average size even the smallest of these figures. It should be specially noted that Calkins does not claim that in changing from the number of micro-nuclei characteristic of aurelia (two) to that characteristic of caudatum (one) there was an accompanying change to a larger size, as would be expected if this were an actual change from one type to the

other. On the contrary, there was a reverse change, to a somewhat smaller size (p. 6), though the decrease in size is plainly such as might be expected from the increased rate of fission at that time.

Thus there appears to be no evidence that Calkins has ever had under examination representatives of the real *Paramecium aurelia*, as understood by Maupas, Hertwig, and the present paper. It must be concluded that Calkins expresses the real state of the case when he says "It may be that my observation was made on a chance abnormality,<sup>2</sup> that paralleled *P. aurelia* and that the real *P. aurelia* retains its integrity as a species" ('06, p. 8). Further evidence of this will be given in Part 2 of this paper, where it will be shown that the two species are constant (at least for long periods), and that they differ in other respects besides the micronuclei.

Thus in reading Calkins' recent textbook ('09) and his later papers, the reader who desires to understand the relation of the facts brought out to these two existing types (or species) will do well to substitute throughout the specific name *caudatum* Ehr. for that of *aurelia* Müll.

Naturally the question comes up: How does such a condition as the presence of an extra micro-nucleus or the absence of one of the typical ones arise? In a single individual of the largest race *L*<sub>2</sub> there was found a condition which may possibly throw some light on the point. Fig. 11 shows this individual as it appears upon the slide; fission was nearly completed when the animal was killed. The micro-nucleus in the posterior half (left in the figure) appears typical in every respect, but that in the anterior half is very unusual in appearance. At this stage of division the daughter micro-nuclei are usually condensing or contracting into the rather small resting condition. But this one is extended into a spindle shape as though in preparation for another division. If such a division took place we should, of course, have one of the daughter cells with two micro-nuclei instead of with the normal number, one. But it is possible that there is here represented only a very great retardation in the assumption of the typical condition.

<sup>2</sup> Of *P. caudatum*.

A point of some interest, though not directly connected with the matter of distinctness of the two groups, was raised by Simpson ('01) who was not able to determine whether in aurelia forms "the two halves of the same micro-nucleus go to one daughter, or whether it is a half of each of the micro-nuclei that go to form the daughter micro-nuclei in any one of the offspring." Fig. 4 is a drawing of a section of an individual of an "aurelia" race. The section though somewhat oblique is fortunately cut exactly in the plane of the dividing micro-nuclei and shows all but a very small fraction of the entire division figures in a single section. Other cases were not obtained in which the entire process was visible in a single section, but it was not difficult to find many instances in which the connections of the micro-nuclei could be accurately determined and followed through the two or three sections bearing them. It is thus perfectly clear that in the "aurelia" forms a half of each micro-nucleus goes into each of the daughter cells.

Thus a cytological study shows that there are two groups of races of these elongated *Paramecia*, differing absolutely in the structure of the micro-nuclei, and differing typically (though with some rare exceptional individuals) in the number of the micro-nuclei. The larger races have typically one micro-nucleus, of the structure and size shown in fig. 8; these races have commonly been grouped as *Paramecium caudatum* Ehr. The smaller races have two micro-nuclei, of the structure shown in fig. 10; these have commonly been grouped as *Paramecium aurelia* Müller. Even in the rare individuals where the number of micro-nuclei is not the typical, the structure of the micro-nucleus is always that which is typical for the race; with an alteration in the number of micro-nuclei there is no alteration in the structure. Thus there is no overlapping of the two forms, no transformation from one to another, so far as the structure of the micro-nuclei is concerned. Thus there is cytological warrant for distinguishing caudatum races from aurelia races, and it seems probable that it will continue to be convenient to designate these as two species. No useful purpose appears to be served by adopting the circumlocution "caudatum form of *Paramecium aurelia*" in place of "*Paramecium*

caudatum," and if this circumlocution implies that one has been shown to change into the other, it is actually misleading.

## PART 2. OTHER CHARACTERISTICS OF THE DIVERSE RACES

H. S. JENNINGS

### 1. *Differences in size and their permanence*

The two pure lines or races that have been longest in the laboratory are those designated by *D* and *c* in the earlier paper (Jennings, '08). Each was derived from a single individual; *D* from a large specimen isolated April 12, 1907, *c* from a small specimen isolated April 9, 1907. These have thus been in the laboratory at the present time (June, 1910) more than three years. At the first measurement the progeny of *D* had a mean length of 182.76 microns; those of *c* a mean length of 130.12 microns (l.c., pp. 404, 405). Throughout the paper of 1908 many measurements of *D* and *c* are given, taken at intervals from June 11, 1907, to March 19, 1908. The length for *D* was found to vary under different environments, from 146.11 to 202.28 microns; that for *c* from 99.67 to 158.8 microns. Under the same environment the race *c* always ranged about 50 microns shorter than the race *D*. The most recent measurement of these two races, taken April 21, 1910, more than three years after they were first isolated and after they had been kept for seven weeks with the greatest care under identical conditions, give *D* a length of 162.28 microns; *c* of 109.25 microns. Thus the difference between the two races is quite permanent. *D*, as we have seen, belongs to the caudatum group, having a single micro-nucleus; *c* to the aurelia group, with two micro-nuclei.

Measurements of other races at long intervals show similar constancy in the differences in size, even though the animals are kept for long periods with the most rigid care under the same conditions, nutritive and otherwise. This is as true of the races distinguishable within each of the two main groups (caudatum or aurelia), respectively, as of those belonging to different groups. This may be shown in brief in the following table, giving the measurements at diverse periods of the races most studied. Where the

same date is given for different races, it will be understood that those races had been kept for many generations under identical conditions. This is particularly true for the last date given, April 21, 1910. In this case all the races measured had been kept, for other purposes, under rigidly identical conditions, for a period of seven weeks; these measurements therefore probably give a very correct idea of the relative sizes of the races. It has been thought best to include in this table, for reference, the measurements of most of the lines on which work was done, even though two lines may belong to the same race, so far as size is concerned.

TABLE 1

Measurements of diverse pure lines of *Paramecia* at different dates. Each line designated by a number or letter consists of the progeny of a single individual (or in some cases of a single pair). The lengths given are the means of fifty to a hundred individuals, and have a probable error in each case of less than two microns.

RACES	AVERAGE LENGTH IN MICRONS						
	June 11, 1907	Nov. 23, 1907	Feb. 5, 1908	Feb. 26, 1908	Mar. 13, 1908	Mar. 19, 1908	Apr. 21, 1910
(Caudatum group)							
<i>L</i> <sub>2</sub>				206.4	220.6		176.8
<i>G</i> <sub>1</sub>				201.4	211.0		
<i>A</i> <sub>1</sub>				193.6	203.6		
<i>20</i>							180.0
<i>D</i>	188.4		169.8	176.9	187.0		162.3
(Aurelia group)							
<i>k</i>				125.9		125.0	125.0
<i>C</i> <sub>2</sub>				128.9		119.2	125.8
<i>g</i>		129.3	114.7	124.4		140.8	
<i>c</i>	130.1		99.7				109.3
<i>i</i>		88.3	93.6				89.6

The table gives measurements of five lines (each derived from a single individual or pair) in each of the two groups.<sup>3</sup> But some of these lines evidently belong to nearly or quite the same race, so far as size is concerned. Three races clearly distinguished by permanent differentiations in size are shown in both the caudatum and aurelia groups. In the caudatum group the largest race is represented by  $L_2$  and 20, the next by  $A$ , the smallest by  $D$ . In the aurelia group the largest race is represented by  $C_2$ ,  $k$ , and  $g$ ; the next in size by  $c$ , the smallest by  $i$ . In the paper of 1908 a race  $Nf_2$ , intermediate in size between the caudatum and aurelia groups, was described. Unfortunately this race was lost before its other characteristics could be studied. Fig. 12 shows the characteristic relative mean sizes of a number of different races.

The measurements given in table 1 are the means for cultures as a whole, including young and old; they doubtless give nearly the dimensions of adults. These data were supplemented by measurements of the various races under identical conditions taken at a given age. The age selected was ten minutes. The measurements are thus for very young individuals, and are therefore less than those of table 1, but they should of course show equally the diversities of the races.

The measurements were made in the following way. The animals examined had (save in case of the line  $c$ ) been living in " $\frac{1}{2}$  standard" hay infusion, regularly changed, for more than two months just prior to the taking of the measurements, so that they were in similar nutritive conditions. The race  $c$  was kept under the same conditions as the others for a week before the measurements, so that it was doubtless in similar condition. The day before making the measurements all were transferred to fresh " $\frac{1}{2}$  standard" hay infusion. The next day many were found to be dividing. Dividing specimens were isolated, and the moment of separation observed. The animals were then kept to the age of

<sup>3</sup> The races  $G_1$ ,  $A_1$  and  $g$  are included in the table, although they were lost before the number and structure of the micro-nuclei was determined. But from their close resemblance to known races, there can be little doubt that  $G_1$  and  $A_1$  belong with caudatum,  $g$  with aurelia.

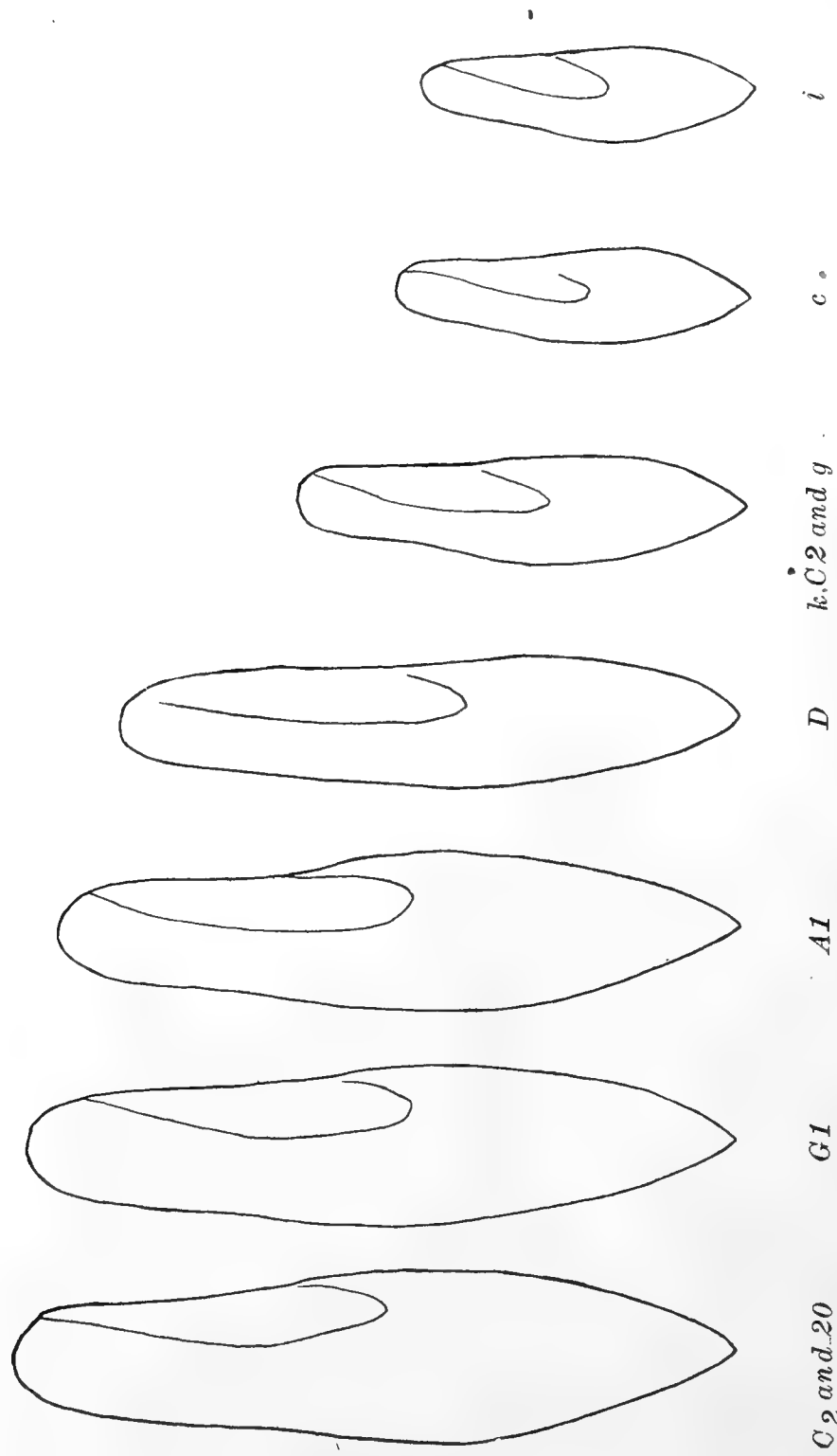


Fig. 12. Diagram showing characteristic average sizes of the diverse races, at a magnification of 375 diameters. These outlines are not camera figures of actual individuals, but are constructions from measurements. The designations of the races used in this paper are given beneath the corresponding outlines.

10 to 11 minutes, killed in Worcester's fluid, and measured.<sup>4</sup> Ten to fifteen specimens of each race were measured at this age.

The mean length and breadth for the different races examined are given in table 2.

TABLE 2

Mean length and breadth in microns for a number of diverse races at the age of ten minutes.

Race	Number measured	Length	Breadth
(A. Caudatum)			
<i>L</i> <sub>2</sub>	15	144.13	36.27
<i>20</i>	14	131.71	45.86
<i>D</i>	13	135.08	39.39
(B. Aurelia)			
<i>k</i>	10	103.00	30.20
<i>C</i> <sub>2</sub>	15	100.40	29.74
<i>c</i>	15	86.27	28.27
<i>i</i>	15	75.60	25.34

On comparing the lengths given in this table with those given in table 1, it is evident that the races show at the age of ten minutes the same order of relative size as in the samples taken from extensive cultures including all ages, but with one apparent exception. The race *20* falls below *D* in length in the present table, whereas in table 1 it stands notably above it. But this peculiarity of race *20* is fully explained by the facts observed in taking the measurements. Twenty-four hours after the animals were placed in fresh " $\frac{1}{2}$  standard" infusion there were no dividing specimens in race *20*, though there were in all the other races. A repetition of the experiment gave the same result. It was found that the only way to get dividing specimens of race *20* was to examine the culture several hours earlier than in the case of the other races. Thus the dividing specimens of *20* were taken but about *eighteen* hours after their introduction into the fresh infusion, the other

<sup>4</sup> The age ten minutes was selected for comparison with the measurements of Popoff ('09) at that age, in studying the relative volume of nucleus and cytoplasm. It had been my intention to make a similar study of nucleus and cytoplasm for the diverse races; but it was found that at this age the nuclei were still very irregular, so that the plan was not carried out. It will be undertaken later.



paces full twenty-four or more hours after. Now, after being placed in the fresh infusion, the animals often shorten and thicken greatly, as demonstrated in an earlier paper (Jennings, '08, p. 472). The race 20 passed into fission while still short and thick, the other races not till they had become thinner and more elongated again. The table shows that race 20 was not only shorter than would be

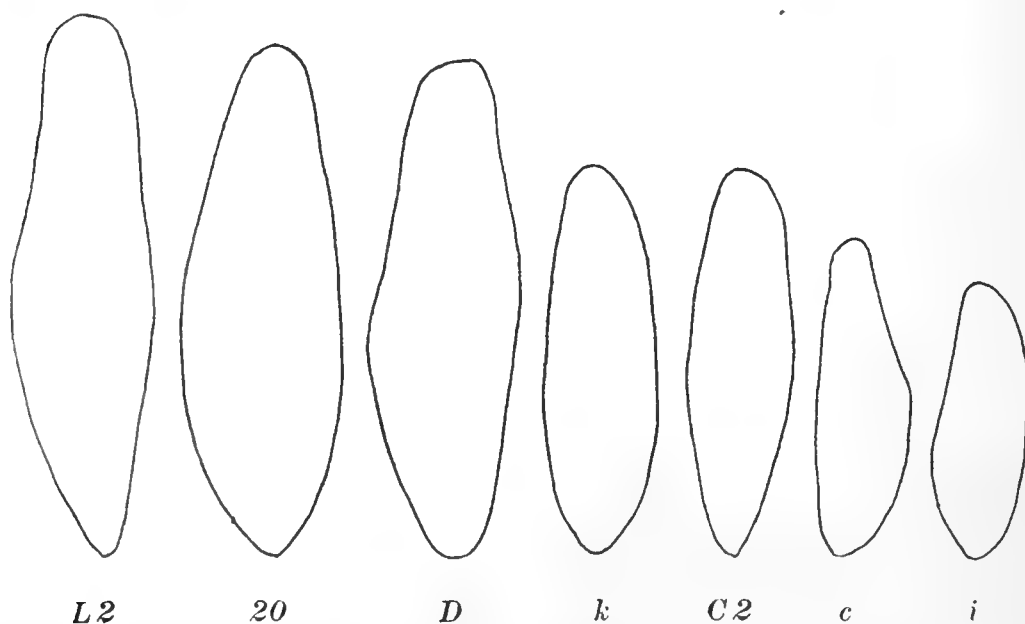


Fig. 13. Characteristic outlines of individuals of certain races at the age of ten minutes, showing relative sizes. Drawn with Edinger drawing apparatus.  $\times 375$ .

expected, but also much thicker. Properly, therefore, race 20 does not belong in the table, since the conditions for it were different from those for the other races.

These facts are an illustration of the physiological differences between races, to be discussed later. In mean adult size, as we have seen, race 20 does not differ appreciably from race  $L_2$ ; but in this matter of the conditions under which fission occurs there is a marked diversity between the two.

Fig. 13 shows characteristic relative sizes in a number of different races at the age of ten minutes.

To test accurately the relation of these diversities in size to environmental conditions, a number of cultures were set in progress

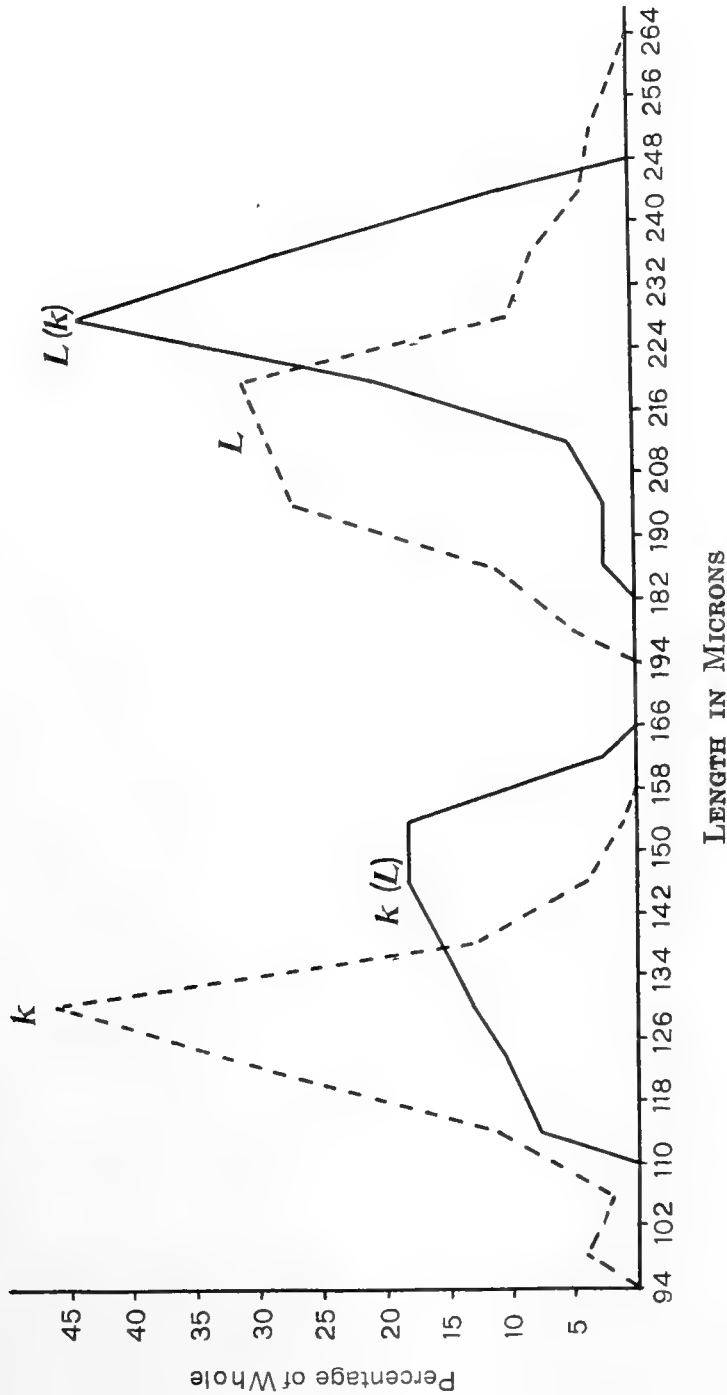


Fig. 14. Polygons of variation in length for a culture of the races  $k$  and  $L_2$ , after they have been living together for five months; together with the polygons for the two races taken separately. The broken lines, marked  $k$  and  $L$  show the polygons for the two races when cultivated separately (100 specimens of each measured February 26, 1908). The continuous lines show the two distinct polygons obtained when a random sample of the mixed culture is measured (March 7, 1909),  $k(L)$  showing the  $k$  component,  $L(k)$  the  $L_2$  component.

containing two diverse races easily distinguishable by their sizes. Such mixtures were made of  $L_2 + k$ ;  $L_2 + i$ ;  $D + c$ ;  $D + i$ ;  $k + i$ , and  $C_2 + i$ . The mixed cultures were kept for months. The two component races remained in every case quite distinct in spite of the common environment. They could easily be distinguished when examined living with low powers of the microscope, and careful measurements and drawings of preserved material demonstrated the presence of the two races clearly. In the following are given some of the facts and results of specific experiments of this sort.

On October 8, 1908, specimens of  $L_2$  and  $k$  were placed together in the same culture. Previous measurements had shown that when these two races are bred separately under as nearly as possible the same conditions, the range of variation for  $k$  is from about 96 to 160 microns, with maximum at 128, while for  $L_2$  the range is from 176 to 264 microns, with maximum at 208 microns (measurements of February 26, 1908). The polygons of variation for these two are shown in fig. 14, at  $k$  and  $L$ .

On November 6, after the two had been living mixed together for about a month, a sample of 154 specimens showed a range of variation from 104 to 264 microns, with two maxima at respectively 140 and 224 microns, showing that the two races were still present. On March 7, 1909, five months after the two races were mixed, another random sample of 77 specimens was measured. The range of variation was now from 112 to 236 microns, with two maxima at respectively 132 and 220 microns. Fig. 14 shows the polygons of variation for this mixture, in comparison with those for the two races taken separately.

An examination with the microscope showed clearly the individuals of the two races, differing widely in size. Fig. 15 shows a collection of individuals of the two races, which had been living together in the same culture for five months. These illustrate a number of points to which we shall later refer in giving an account of the structural features of the two races.

In cases where the two races mixed belonged to the same group (caudatum or aurelia) the results were the same. The two races remained quite distinct. A number of such mixtures were made of

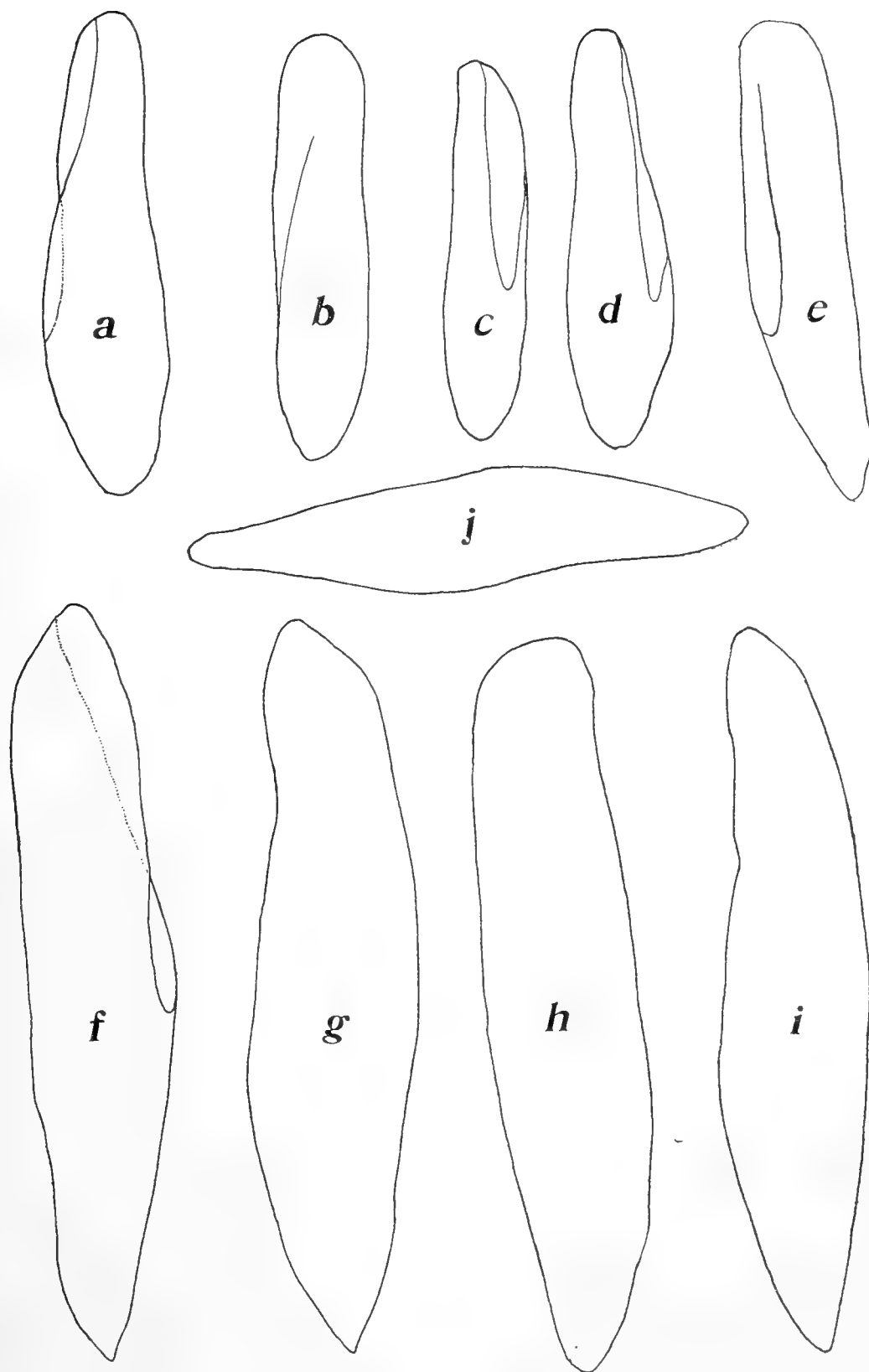


Fig. 15. A collection of individuals from a culture in which the small race *k* and the large race *L*<sub>2</sub> have been living together for five months (October 8, 1908 to March 9, 1909), showing that the two races are still distinct. The upper row (*a* to *e*) shows individuals belonging to *k* (aurelia); the lower row (*f* to *j*) individuals of *L*<sub>2</sub> (caudatum). (The specimen marked *j* is a starved individual of *L*<sub>2</sub>). Note the difference in the form of the posterior part of the body in the two races. Edinger drawing apparatus.  $\times 375$ .

$k + i$ , and of  $C_2 + i$ , both belonging to the aurelia group, but differing in mean size.

Taking, for example, the case of  $k + i$ , previous measurements had shown the mean length of  $k$  to be about 125 microns; that of  $i$  to be about 93 microns. (See table 1.) Fig. 16,  $k$ ,  $i$ ,

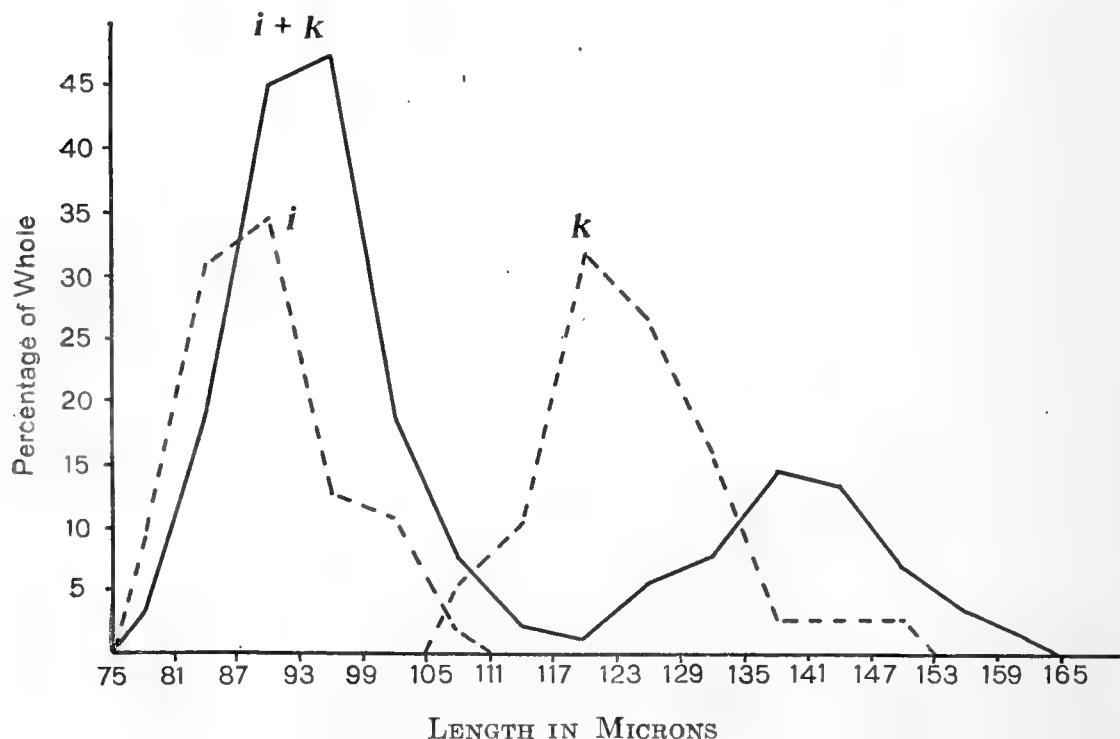


Fig. 16. Polygons of variation in length for a culture in which the two aurelia races  $i$  and  $k$  have been living together for two weeks, in comparison with polygons for each race taken separately. The broken lines are for the two separate races (measurements of April 21, 1910); the continuous line shows the double peaked polygon from the culture in which both are present (measurements of June 13, 1910).

(In the mixed culture the race  $i$  was more numerous than  $k$ , so that the left peak, representing  $i$ , is higher than the one to the right, representing  $k$ . Further, the nutritive conditions in the mixed culture were such that both races show a greater mean length than in the separate cultures, thus displacing the peaks to the right.

shows polygons of variation of  $k$  and  $i$  taken separately, while  $k + i$  shows the polygon of variation formed when the two have been living in the same culture for two weeks. The two races are evidently still present. They were found to persist side by

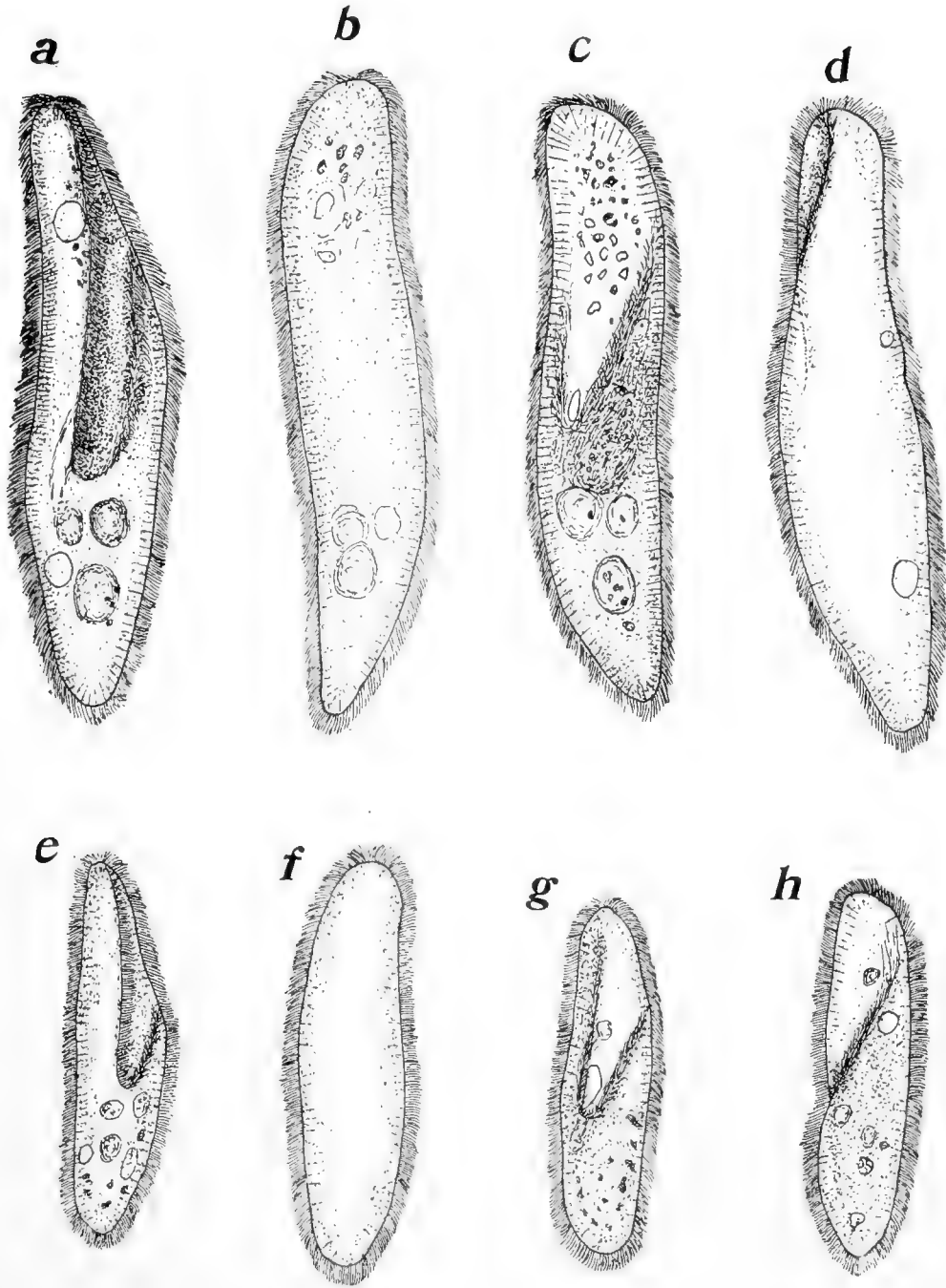


Fig. 17. A collection of individuals from a culture in which the two aurelia races *i* and *k* have been living together for three weeks, to show the differences between the two races when living in the same medium. The upper row (*a* to *d*) shows characteristic individuals of *k*, the lower row (*e* to *h*) individuals of *i*. *a* and *e* show a dextro-ventral view; *b* and *f* the dorsal surface; *c* and *g* the ventral surface. *d* shows the left side; *h* the sinistral-ventral surface. *a* and *c* are different views of the same individual; note the difference in form. Edinger drawing apparatus.  $\times 375$ .

side for months. Fig. 17 shows typical specimens of the two races from a culture in which they had been living together for three weeks.

Mixtures of other races, of  $D + i$ , of  $D + c$ , of  $C_2 + i$ , of  $L_2 + i$ , were made and the progress of the cultures observed for weeks or months. In every case the two races remained evidently distinct; in an examination under the microscope the two diverse sizes were very noticeable. It was not thought necessary to make precise measurements and figures, save for the two typical mixtures above described.

A question that perhaps requires brief discussion is that regarding the relation of these diverse sizes to conjugation.<sup>5</sup> Is it absolutely clear that these "races" of diverse size are not really diverse merely because they represent different periods in the life cycle, from conjugation to conjugation? To this question an affirmative answer can be given; it is absolutely clear that the differences are not due to the period of the cycle, for *many of the races have gone through several or many complete "cycles" in the laboratory, without changing their relative sizes.* The race  $k$  has gone through at least twenty such cycles; the races  $C_2$  and  $g$  through several; the races  $c$  and  $L_2$  through two; the race  $i$  through at least one—and at no time has there been any change in the relative size of these races. Fig. 18 shows the relative sizes of certain races at conjugation, when living in hay culture. The pairs of  $k$  and  $i$  in this figure were living together in the same culture; they therefore show the relative size at conjugation under identical conditions.

It is clear therefore that the differences in size among the different races are independent of environmental influence—the differences persisting when the organisms are kept for long periods in the same environment. It is further clear that the differences are permanent, at least for a period exceeding three years, and that they are not dependent upon the relative period in the life cycle of the animals from conjugation to conjugation.

<sup>5</sup> This question was taken up in my paper of 1908; further data are here added.

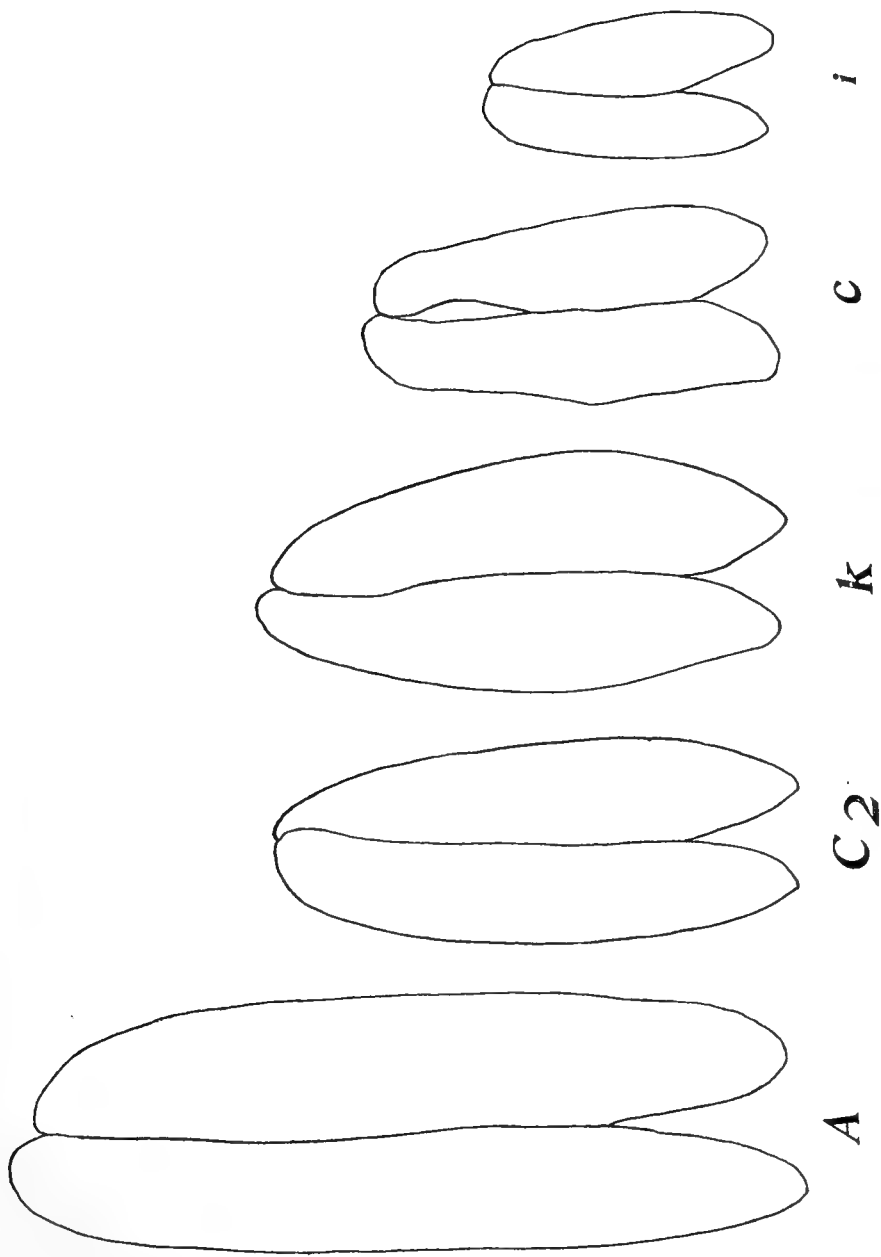


Fig. 18. Relative sizes of a number of races at the time of conjugation, when living in hay cultures. *A* is a large caudatum race; the others are the aurelia races described in this paper. Edinger drawing apparatus.  $\times 375$ .



2. *Similarities and differences of the various races in form and structure*

We have seen that the various races show permanent differences in size, and that certain races (*aurelia*) differ in the number and structure of the micro-nuclei from others (*caudatum*). Are the races identical in other respects? Are the small races merely miniature replicas of the larger ones, so far as form and structure are concerned?

As is well known, certain diversities have been set forth as distinguishing the species *caudatum* from the species *aurelia*. Ehrenberg's original description of *caudatum* as a separate species ('38) was based on a difference in form, the posterior part of *caudatum* was said to be longer and more slender than in *aurelia*, tapering (as shown by his figures) regularly backward from about the middle to the posterior point. In *aurelia* on the other hand the posterior half of the body is shown as more swollen and less pointed. Schewiakoff in his excellent monograph of the *Holotricha* ('96) says that in *P. aurelia* the spindle shaped body is equally narrowed toward both rounded ends, whereas in *P. caudatum* the anterior part of the body is but slightly narrowed, being almost cylindrical in form, while the posterior part is much narrowed, so as to be conical in form, though rounded at the tip. Moreover, *P. caudatum* is said to have at the posterior end a bundle of longer cilia, which is lacking in *P. aurelia*. These differences are clearly shown in Schewiakoff's figures. Let us examine our various races with these and similar points in mind.

*Form.*—It is difficult to make any precise statements that will hold generally with regard to characteristic differences in form between the *caudatum* and *aurelia* groups. It is not difficult to find specimens of *caudatum* with the body broad in front and drawn to a long point behind (for example, fig. 15, *f* or *h*), as described by Ehrenberg and Schewiakoff; nor of *aurelia* with the spindle-like form described by Schewiakoff (for example, fig. 17, *a* or *d*). But it is equally easy to find specimens of *aurelia* with the supposed *caudatum* form (as fig. 15, *e*, fig. 17, *b*), and specimens of *caudatum* with the supposed *aurelia* form (as fig. 15, *i*).

Furthermore, it is easy to find specimens of either group that do not show the forms described as characteristic for either (see fig. 20, 22); it is indeed perhaps hardly proper to try to discuss in any precise way the forms of these animals without taking into consideration the environmental conditions, since these change the form enormously. After working with the animals continuously for some years and drawing great numbers with the camera, I find that I carry away the impression, however, that there is a real difference between the two groups; that in the caudatum races the posterior half of the body is as a rule relatively more long and slender than in aurelia, so that the total breadth of the animals is a little less in proportion to the length, and that the posterior tip in caudatum is more commonly sharply pointed than in aurelia. An idea of what is meant will be gained by comparing fig. 15, *f* (caudatum) with fig. 15, *a* (aurelia). But it would be easy to point out cases in which these differences do not hold, as a glance at the figures accompanying this paper will show. The differences if they are real are therefore of what might be called a statistical character; they would come forth only on comparing large numbers of individuals. In a former paper (Jennings, '08), it was shown that there *is* such a statistical difference in regard to one of the points mentioned; from measurements of very large numbers of individuals under all sorts of environments it was shown that the aurelia races average a little broader in proportion to the length than do the caudatum races (p. 501). But individuals of any race of either group may under the influence of varied environments take on forms either much more slender than that characteristic of caudatum (as in fig. 15, *j*), or much plumper than that characteristic of aurelia (as in fig. 22).

To further test these points, careful measurements were made of representatives of the two groups that had been living for a month together in the same culture. On October 8, 1908, a mixed culture was made containing the race  $L_2$  (caudatum) and  $k$  (aurelia.) On November 6, samples from this culture were killed, and twenty-five representatives of each race were subjected to very careful measurements, in order to determine (1) whether the relative breadth differs even when the animals are living in the

same environments; (2) whether the form of the posterior half of the body differs in the two races.<sup>6</sup>

The method of measurement was as follows: Twenty-five specimens of each race, taken at random, were outlined at a magnification of 500 diameters, by the use of the Edinger projection apparatus. The following dimensions were then taken from these outlines: (1) total length; (2) greatest breadth; (3) breadth at the middle; (4) breadth at certain distances behind the middle, the distances selected being respectively  $\frac{1}{5}$ ,  $\frac{2}{5}$ ,  $\frac{3}{5}$  and  $\frac{4}{5}$  of the distance from the middle to the posterior end (the greatest breadth in most cases coincided with the breadth at the middle).

It is evident that the breadths measured in the posterior half of the body will enable us to determine whether caudatum tapers backward more rapidly from the middle than does aurelia, as has been supposed to be the case. This is done by reducing the breadths found at the intervals behind the middle to percentages of the breadth at the middle. These percentages of course decrease more rapidly backward in the animals that taper more rapidly backward.<sup>7</sup>

As to the relative breadth, in proportion to the length, the mean ratio of greatest breadth to greatest length<sup>8</sup> for the twenty-five specimens of *L*<sub>2</sub> (caudatum), was 21.9 per cent; for the twenty-five specimens of *k* (aurelia) 24.3 per cent. A similar result was reached later when the race *L*<sub>2</sub> (caudatum) was compared with the very small aurelia race *i*, living in the same culture, under conditions of excessive nutriment (see p. 527). Here twenty-five specimens of *L*<sub>2</sub> gave a mean ratio of breadth to length amounting to 34.1 per cent while twenty-five *i* gave a

<sup>6</sup> As we have before mentioned, the two races could easily be distinguished, even though living together, by the great difference in size. See p. 516.

<sup>7</sup> Owing to the fact that the apparent shape of the anterior half of the body differs, depending on how the specimens happen to lie (as shown later), characteristic differences between the two species in the anterior half, if such exist, cannot readily be tested in this way.

<sup>8</sup> These mean ratios are of course obtained by dividing for each specimen separately the breadth by the length, and taking the mean of the ratios so obtained. Dividing the mean breadth of all by the mean length of all would give a different result.

mean ratio of 38.1 per cent. Both these determinations confirm the previous general result, and show that even when living in the same culture the aurelia races are proportionately a little broader than the caudatum races.

As to the rate of taper from the middle backward, the data are given in table 3.

TABLE 3

Ratio of breadths at certain distances behind the middle to breadth at middle, in 25 specimens of each race living in the same culture.

DISTANCE BEHIND MIDDLE	MEAN RATIO TO BREADTH AT MIDDLE	
	<i>L</i> <sub>2</sub> (caudatum)	<i>K</i> (aurelia)
$\frac{1}{5}$	94.2	97.8
$\frac{2}{5}$	86.2	92.0
$\frac{3}{5}$	72.3	85.5
$\frac{4}{5}$	49.6	54.2

The table shows that the relative breadths at the regions measured in the posterior half of the body are throughout less in the caudatum race than in the aurelia race, thus demonstrating that caudatum does taper more rapidly backward than aurelia, and confirming the descriptions of Ehrenberg and Schewiakoff on this point.

If we construct from these data the typical dimensions for the two races when living under these conditions, we find them to be as shown in table 4. Here the mean length and mean breadth

TABLE 4

Typical mean dimensions in microns of the races *L*<sub>2</sub> (caudatum) and *k* (aurelia) living in the same culture.

	<i>L</i> <sub>2</sub>	<i>K</i>
Length.....	229.7	143.9
Breadth at middle.....	49.7	34.5
Breadth $\frac{1}{5}$ behind middle.....	46.8	33.8
Breadth $\frac{2}{5}$ behind middle.....	42.8	31.7
Breadth $\frac{3}{5}$ behind middle.....	25.9	29.5
Breadth $\frac{4}{5}$ behind middle.....	24.6	18.7

at middle are the actual means for the entire number, while the other breadths are calculated from the mean breadth at the middle by use of the ratios given in table 3. If now we construct figures with these dimensions, we have in fig. 19 the typical forms and

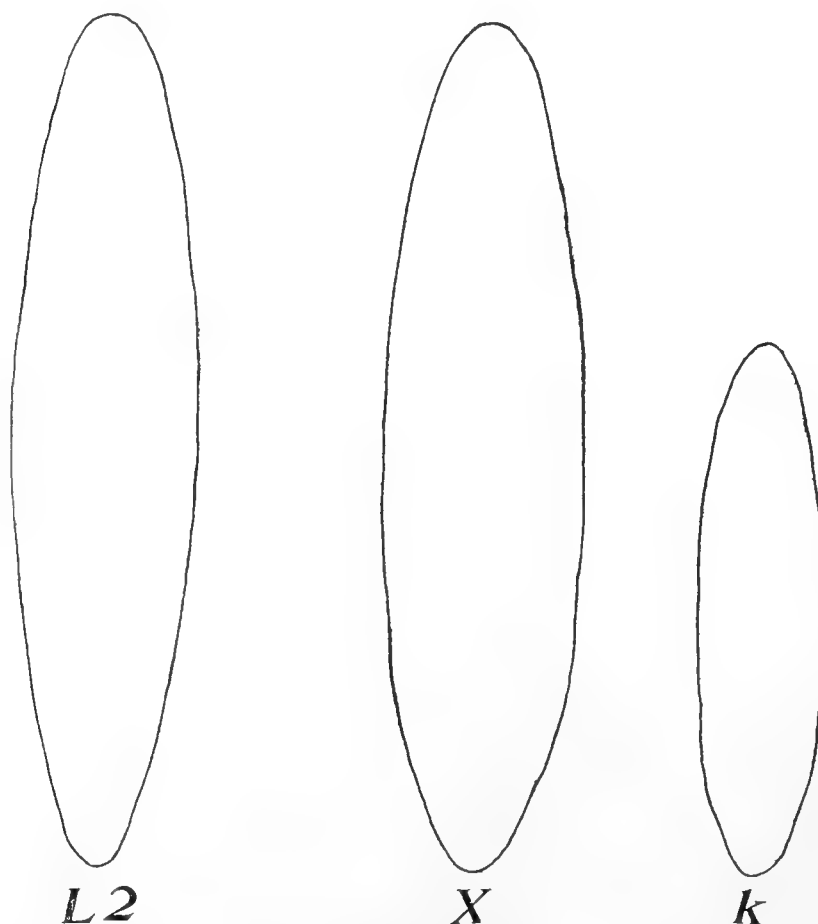


Fig. 19. Characteristic differences in form between caudatum ( $L_2$ ) and aurelia ( $k$ ) when living together in the same medium. The forms and sizes shown are the averages from twenty-five specimens of each. X shows the form of  $k$  (aurelia) when magnified to the same size as the caudatum race. Note the greater breadth and different form of the posterior part of the body in aurelia. The magnification (for  $L_2$  and  $k$ ) is 375.

sizes of the races  $L_2$  and  $k$  when living together under the conditions of the culture studied. Fig 19 also gives at X the form and proportions of  $k$  as it would be if it were magnified till the length was the same as for  $L_2$ ; comparison of this with  $L_2$  brings out clearly the differences in form.

The account just given deals with two races under such nutritive conditions that such were slender. A further comparative study of two races was made under such conditions that both were thick and plump. For this study the caudatum group was

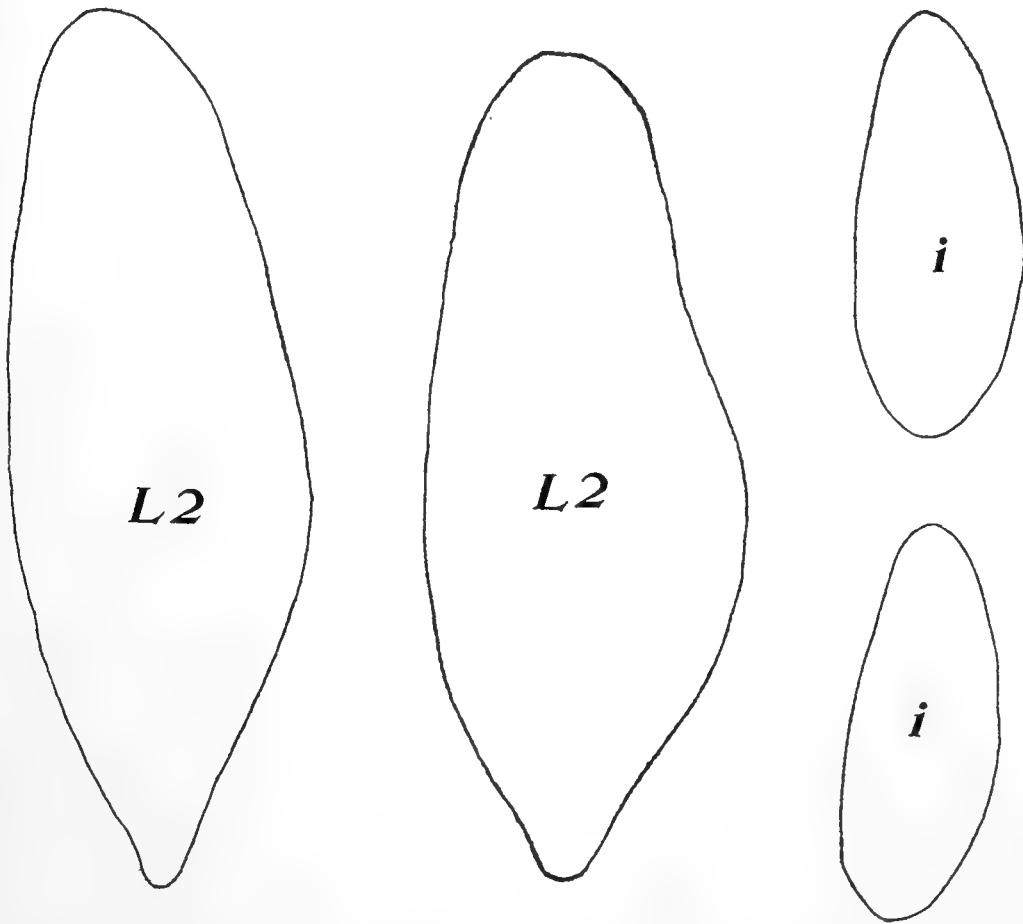


Fig. 20. Characteristic forms and sizes of  $L_2$  and  $i$  when both are extremely plump. Edinger drawing apparatus.  $\times 375$ .

again represented by the race  $L_2$ , while the aurelia group was represented by the very small race  $i$ . To the culture containing both these races a quantity of bread was added; this induced the animals to become very stout. Twenty-four hours after the introduction of the bread a sample was killed, and twenty-five of each of the races were outlined with the projection apparatus, as before. The difference between the two races in form was much more noticeable when the animals were plump, so that measurements were not required to show it. Fig. 20 illustrates very clearly the difference.

In *i* (aurelia) the posterior part of the body hardly tapers backward at all, and the posterior end is smoothly rounded, whereas in *L*<sub>2</sub> (caudatum), the posterior region tapers sharply backward and the posterior point persists even when the body becomes very thick; it stands out as a marked protuberance. In no case was such a protuberance present in the specimens of aurelia. Besides this difference, the specimens of the caudatum race were more slender than those of the aurelia race. In the former the breadth was 34.1 per cent of the length; in the latter 38.1 per cent.

No characteristic differences in form have been noticed between the races of different size belonging to the same group or species (caudatum or aurelia), though much attention has been directed to this point. A study of characteristic forms of the large aurelia race *k* and of the small aurelia race *i*, under identical conditions, is given in figs. 21 and 22.

Within any single race the form of the anterior end differs, depending on whether one sees it from the side, or dorso-ventrally. As seen from the side the anterior end is narrow and pointed (as in Schewiakoff's figure of *P. aurelia*); in dorso-ventral view it is broad and blunt (as in Schewiakoff's figure of *P. caudatum*). These differences are well brought out in the two views of a single individual of *k* (fig. 17, *a* and *c*), of *i* (fig. 17, *g* and *h*); they are well seen in *L*<sub>2</sub> by comparing *h* and *i*, fig. 15. The differences between dorso-ventral and lateral views become much less marked when the animals are very plump, as in fig. 20.

The posterior part of the body is usually circular in section, but when the animals are starved, they become greatly flattened dorso-ventrally, as illustrated in the two views of an individual of the race *c* given in fig. 23. Often under these conditions the animals become ridged and folded, so as to be quite irregular in section.

*Posterior tuft of long cilia.*—This has commonly been set forth as a distinctive character of caudatum, but if there is any distinction in this respect, the difference is extremely difficult to detect. No matter how carefully killed, a large proportion of the individuals in any race will not show this posterior tuft of long cilia, owing doubtless to the irregular and entangled positions of

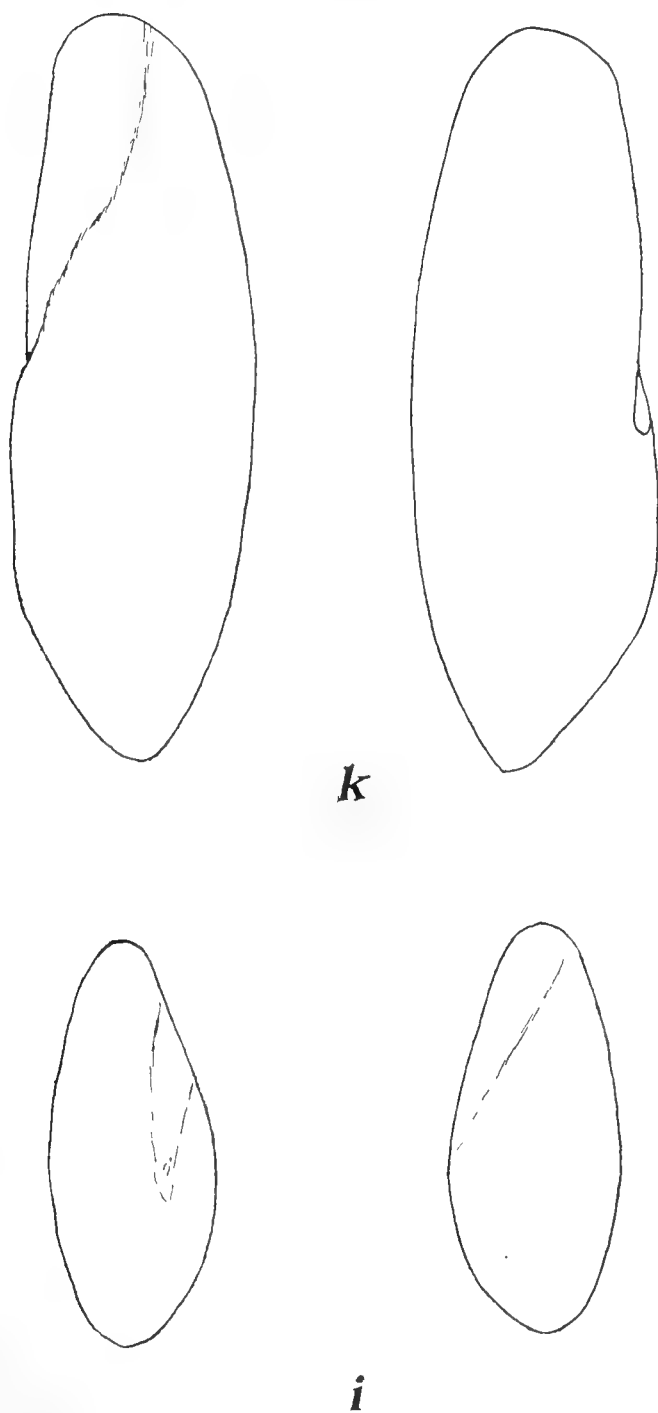


Fig. 21. Form and size of races *k* and *i* (both aurelia) under conditions of high nutrition. The two had lived together in the same culture for one month; twenty-four hours before the figure was made some bread was added to the culture. Edinger drawing apparatus.  $\times 375$ .

the cilia at the time of death, as well as to the discharge of the trichocysts. Some individuals however show it, and this was true



in all the races examined,  $L_2$ ,  $D$ ,  $21$ ,  $43$ , of the caudatum races, and  $k$ ,  $C_2$ ,  $c$  and  $i$  among the aurelia races. This characteristic is not marked nor easily detected in any case. Since the posterior

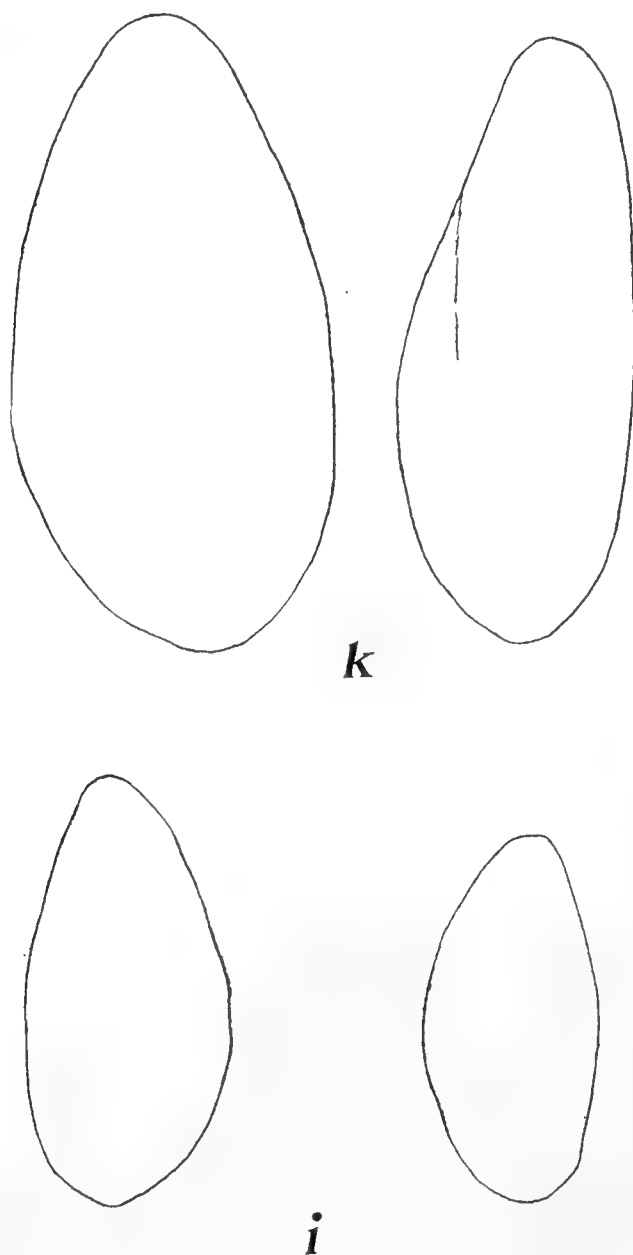


Fig. 22. Form and size of races  $k$  and  $i$  (both aurelia) under conditions of excessive nutriment. The culture is the same as that of fig. 21, but after four days in the bread infusion. Edinger drawing apparatus;  $\times 375$ .

tip is in the caudatum races more often sharply pointed than in the aurelia races, its bundle of cilia more often stands out clearly

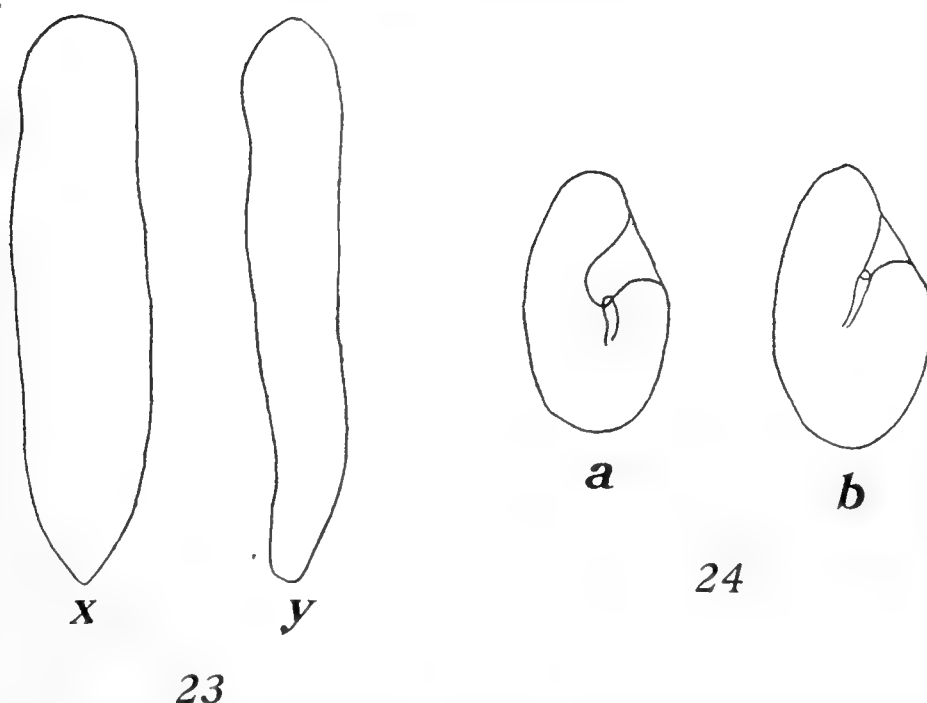


Fig. 23. Dorsal (*x*) and lateral (*y*) views of a starved specimen of the race *c* (*aurelia*), showing how under these conditions the posterior part of the body is compressed dorso-ventrally.  $\times 375$ .

Fig. 24. Outlines of *Paramecium bursaria* (*a*) and *P. putrinum* (*b*), to show the form. From the figures of Schewiakoff (1896).  $\times 225$ .

in the former case; the supposed difference between the two species in this respect is probably to be accounted for in this way.

(In preserved specimens, in many cases a few trichocysts have been discharged from the posterior tip only, extending merely some distance beyond the cilia; this is especially likely to happen in races of *caudatum*. They then give the appearance of a very pronounced bundle of long cilia in this region. Comparative study will show however every gradation between such cases and those in which the trichocysts have extended many times the length of the cilia, so as to be at once recognizable as trichocysts.)

*Trichocysts*.—It is said that in the species *P. bursaria* the trichocysts are sometimes absent. It is worth noting therefore that in all the races mentioned in this paper trichocysts are present. Weak methylene blue readily causes their discharge; and the discharge usually occurs to a certain extent in killing fluids.

*Contractile vacuoles*.—In *Paramecium putrinum* it is said that there is usually but one contractile vacuole (Schewiakoff, '96,

p. 336; p. 343). It should be mentioned therefore that in all the races here described two contractile vacuoles are present, in the usual position (shown in fig. 17, *a* and *d*).

*Summary.*—Thus it is clear that the races of caudatum do differ from those of aurelia in certain other features besides the size and the number and structure of the micro-nuclei. The differences are mainly these: under the same conditions (1) the individuals of caudatum have a breadth slightly less in proportion to the length; (2) the posterior half of the body tapers more rapidly in caudatum; (3) the posterior point persists in caudatum even when the animals become very plump, while in aurelia under these conditions the posterior point disappears. Among the different races of either group (caudatum or aurelia) no characteristic differences of form were noted.

### 3. *Differences among the diverse races with respect to conjugation*

As I have set forth in a paper dealing with conjugation (Jennings, '10) the different races differ greatly in their readiness to conjugate and in regard to the conditions inducing conjugation. Referring to the paper just mentioned for details, we state here merely the essential facts. In the aurelia group, the race *k* is distinguished for its remarkable readiness to conjugate. More than twenty epidemics of conjugation have been observed in it during the period of two years and four months in which it has been in the laboratory. If it is allowed to multiply slowly for two or three weeks, then a considerable quantity of nutrition is added, so that multiplication becomes rapid, then the nutrition is allowed to fall again, the animals almost invariably conjugate. The races *i* and *c*, on the other hand, when subjected to the same treatment usually do not conjugate; epidemics of conjugation are extremely rare in these races. Although *c* has been in the laboratory for three years and one month, but two epidemics of conjugation have been observed in it, in spite of the fact that extreme efforts have been made to induce it to conjugate more frequently, by the use of the means so efficacious with *k*. In the race *i* but two partial epidemics have been observed, though the same treatment was employed as for *k*. Only a small proportion of the individual's con-

jugated even when a period of conjugation occurred. The races  $C_2$  and  $g$ , of the same size as  $k$ , likewise resemble  $k$  in conjugating readily, though apparently less readily than  $k$ .

In the caudatum group conjugation is throughout not readily induced in animals living in the laboratory. On bringing material from the open, the specimens of caudatum are often found conjugating in great epidemics, a short time after being placed in laboratory conditions. But later, conjugation becomes very rare. The race  $D$  (three years and two months in the laboratory) has never been seen to conjugate, though it has been observed more carefully than any other race, and it has repeatedly been subjected to the same conditions that induce conjugation so readily in  $k$ . The race  $L_2$  has shown two epidemics of conjugation, in the two years and four months in the laboratory. It does not conjugate when subjected experimentally to the conditions that induce conjugation in  $k$ . But one other epidemic of conjugation has been observed in a pure race of the caudatum group, when grown in the laboratory.

The readiness to conjugate shows no uniform relation to the relative sizes of the races. Those which conjugate most frequently are the larger races of the aurelia group ( $k$ ,  $C_2$ ,  $g$ ). The large races of the caudatum group ( $L_2$ ,  $D$ , etc.) and the smaller ones of the aurelia group ( $c$ ,  $i$ ) cannot easily be induced to conjugate.

#### 4. *Differences in rate of fission among the different races*

From March 5 to April 11, 1910 (thirty-seven days), an extensive experiment was carried on for determining the rates of fission in the different races. The conditions were kept as nearly as possible absolutely uniform for the representatives of all the races, and the exact number of fissions each day was recorded.

The details of experimental procedure were as follows: Two or more representatives of each race were isolated on a concave slide, a single individual in each concavity. Each was placed in three drops of "standard" hay infusion, made as follows: One gram of pure Timothy hay (*Phleum pratense*) was boiled for ten minutes in 50 cc. of water. To this was added 50 cc. of tap water,

which had merely been warmed to 50° Cent., in order to destroy any foreign *Paramecia* it might contain. This infusion was used quite fresh, and was made anew each day. The animals do not flourish so well if the fluid is allowed to become twenty-four hours old before use. From March 29 on the infusion was employed at but one-half the concentration mentioned; that is, 200 cc. of water was added to the gram of hay, in place of 100 cc. This " $\frac{1}{2}$ -standard" infusion was found to be on the whole better. Every day the number of fissions during the previous twenty-four hours was recorded, and a single specimen was transferred with a capillary pipette to three drops of the fresh infusion on a clean slide. In order that there might be little danger of losing the series by death of a single individual, from each of the original specimens two lines of propagation were carried on, and in a part of the experiment *four*. Thus from every race there were at all times at least four parallel lines of propagation in progress, and during a part of the experiment there were at least eight lines for each race. In the case of several of the races the number of parallel experiments carried through was considerably greater than this, and the experiments were continued much longer. The slides with the different lines were kept side by side in the same moist chambers so that the conditions were all identical for the different races.

The experiment showed that there are certain characteristic differences in the rate of fission among the different races.<sup>9</sup> The characteristic rate of fission for the different races, in the case of healthy lines that have not recently conjugated (though by no means in a state of "depression") are shown for the entire period of the experiment and for the successive weeks in table 5. The table is based, save in one or two cases specified, on lines that lived throughout the experiment. In each case lines from two individuals at the beginning are employed, and so far as possible two lines from each of these individuals are followed, so that data for four lines for each race appear in this record. In cases where

<sup>9</sup> The results as to other influences affecting the rate of fission, and particularly as to differentiation in this matter within the limits of a single race, will be dealt with in another paper.

TABLE 5

Numbers of fissions per week, in the diverse races of the Caudatum and Aurelia groups, when cultivated under uniform conditions.

RACE	INDIVIDUAL	FISSIONS 1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	FISSIONS IN 5 WEEKS	TOTAL FISSIONS IN 37 DAYS	MEAN NO. OF HOURS BETWEEN FISSIONS
(Caudatum Group)									
$L_2$	$a_1$	11	10	13	{ 10	11	55	58	15.31
	$a_2$	(11)	(13)		{ 11	11	55	57	15.58
	$b_1$	8	9	9	{ 10	10	46	49	18.12
	$b_2$	(9)	(8)		{ 6	9	41	43	20.65
$20$	$a_1$	10	12	12	{ 8	11	53	56	15.86
	$a_2$	(10)	(11)		{ 7	9	50	51	17.41
	$b_1$	11	13	13	9	{ 11	57	59	15.05
	$b_2$	(11)	(11)			{ 11	56	59	15.05
$D$	$a_1$	(11)	(10)	(6)					
	$a_2$	(11)	(11)	(6)					
	$b_1$	8	6	7	4	{ 8	33	36	24.67
	$b_2$	(11)	(8)			{ 8	33	35	25.37
(Aurelia group)									
$k$	$a_1$	11	10	12	10	11	54	58	15.31
	$a_2$	10	10	9	9	11	49	51	17.41
	$b_1$	12	9	10	13	12	56	59	15.05
	$b_2$	11	7	10	11	10	49	52	17.08
$C_2$	$a_1$	9	8	7	{ 8	10	42	44	20.18
	$a_2$	(9)	(8)		{ 6	9	39	41	21.66
	$b_1$	9	{ 6	6	{ 8	11	40	43	20.65
	$b_2$	(6)	{ (7)		{ 5	7	33	34	26.12
$c$	$a_1$	12	13	15	{ 13	12	65	68	13.06
	$a_2$	(12)	(12)		{ 11	11	62	65	13.66
	$b_1$	13	12	15	15	13	68	71	12.51
	$b_2$	13	13	16	13	12	67	71	12.51
$i$	$a_1$	15	10	13	9	{ 14	61	63	14.95
	$a_2$			(13)		{ 14	60	64	13.88
	$b_1$	14	14	15	15	12	70	73	12.16
	$b_2$	13	14	14	15	11	67	70	12.69

The two original individuals of each race are designated  $a$  and  $b$ , respectively, and the two series from each of these are designated  $a_1$  and  $a_2$ ;  $b_1$  and  $b_2$ . Numbers in parentheses are for lines that did not live throughout the experiment. The braces following certain numbers show that the line in question was here divided (owing to death of the other line), so that the two following lines are derived from this one. Thus, in the race  $L_2$ , the line  $a_2$  died after the second week, and was replaced by again taking a specimen from the line  $a_1$ .

records existed for several different lines for each race, there were chosen: (1) the lines that were healthy, not showing frequent deaths during the experiment, (2) the lines in which the fission was most nearly uniform in rate.

A number of important points are brought out by the table.

1. There are differences in the rate of fission between different lines belonging to the same race, and thus derived originally from the same individual. Thus, in the race  $L_2$ , the lines derived from the individual  $a$  show during every week, as well as for the entire period together, a more rapid rate of fission than the lines derived from the individual  $b$ . This inherited differentiation within pure lines is of the greatest interest; it will be dealt with in a separate paper.

2. Within the same line the rate is sometimes very different for a certain period, as a week or ten days, from the rate during the rest of the time. This is much more evident when one inspects a table in which the fissions are recorded day by day. The rate in a given line is there seen at times to drop, remain low for perhaps ten days, then return to the original rate. In most or all of these cases there are evidences of pathological conditions during these periods of lowered rate of fission. Monstrosities appear, and many of the specimens die. Therefore these periods of slower rate are not to be considered as giving the characteristic rate for the race when healthy. In comparing different races, the periods when the rate of fission is high and uniform should be compared. (The influences determining these lasting changes in the rate of fission will be dealt with elsewhere.)

3. More important for our present purposes is the fact that in spite of the fluctuation within a given race, it is evident that *there are marked characteristic differences in rate of fission between different races*. In the aurelia group there are evidently three different sets, so far as rate of fission is concerned. The race  $k$  multiplies at about the same rate as do the characteristic lines of the caudatum group, having as a rule ten to twelve fissions per week, and in the total period of thirty-seven days, from fifty-

one to fifty-eight fissions. The race  $C_2$ , which is of the same size as  $k$ , multiplies throughout the experiment less rapidly than  $k$ , with six to nine fissions per week, and with a total of thirty-four to forty-four in the entire thirty-seven days. The two smaller races  $c$  and  $i$  have a much more rapid rate, dividing eleven to fifteen times per week, with totals in the thirty-seven days of from sixty-three to seventy-three fissions. There is no clear difference in rate of fission between  $c$  (larger) and  $i$  (smaller).

In the caudatum group there appear to be at most but two sets so far as rate of fission is concerned.  $L_2$  and  $20$  show essentially the same rate as  $k$  in the aurelia group, with nine to twelve fissions per week, and forty-three to fifty-nine in the period of thirty-seven days. The race  $D$  appears at first view to have a very different rate of fission, the totals for thirty-seven days being but thirty-five and thirty-six and the rate per week being sometimes as low as four to six. The details of the experiment seem to indicate however that the difference between  $D$  and the other races is rather a matter of lack of adaptation of these specimens of  $D$  to continued existence in the culture medium used, than to a natural difference in the rate of fission. In the first week, three of the lines of  $D$  gave the same rate as did the other races of caudatum (ten to eleven per week), and with two of the lines this continued another week. But now all the lines of  $D$  evidently sickened; monstrosities were produced, the fission became irregular or stopped for long periods, and many of the individuals died. Finally the lines from one of the two original individuals died out completely, while the other was barely saved, the individuals being of swollen irregular form, with many abnormalities and deaths. Further, four other series from  $D$  were later started (April 30) and these all gave during the first two weeks rates of fission of ten to twelve or thirteen per week. Thus the normal rate of fission is apparently the same for  $D$  as for the other races of caudatum. The experiment then shows no racial diversity in rate of fission within the caudatum group, though it shows three diverse sets within the aurelia group.



5. *Differences in cultural requirements, and other physiological differences*

The cultures conducted under uniform conditions for determining the comparative rates of fission, revealed certain differences in cultural requirements, and in general vitality, among the different races. In this matter there were likewise diversities between different lines within the same race (a matter to be dealt with elsewhere), but these did not conceal certain characteristic differences in different races.

Thus in following the descendants of a single individual of the race *k* for sixty-two days, not a single death occurred in the direct series. For forty-one days of this period there were four parallel series, without a single death in any of them. In the progeny of the two typical individuals of this race that were propagated thus for sixty-two days, with eight parallel lines, there were but four deaths in the series. In the race *20*, on the other hand, which was propagated from two individuals under conditions identical with those for *k* and parallel with it, for the first thirty-seven days of the experiment, there were twenty-nine deaths in the period of thirty-seven days. In the race *C*<sub>2</sub> in the corresponding period of thirty-seven days there were in the progeny of the two individuals eighteen deaths in the direct series.<sup>10</sup>

More striking are the cases of the race *D* and *43*. In the case of *D*, all four series began to sicken and produce abnormalities during the second week of the experiment. They remained in this condition, multiplying slowly, till at about the end of the third week the two lines derived from one of the individuals died out, while the progeny of the other specimen dwindled to a single specimen, which did not divide at all for three days. It finally revived and propagated itself, but in an irregular and abnormal way, producing many abnormalities throughout the rest of the experiment. It was apparent that the uniform cultural conditions

<sup>10</sup> In this period of thirty-seven days there were, for each race, four lines of experiments in progress during the first twenty-three days and eight for the last fourteen days. Two additional lines were present for each race, from the fifteenth to the twenty-eighth days of the experiment.

were not adapted to the requirements of this race, though for *k* they were quite suitable.

A still more marked case of the same sort is given by the race 43. This consisted of large animals of the caudatum group, all derived from a single wild specimen. At the time of the experiment it was flourishing in a culture consisting of timothy hay in water. When transferred to the slide cultures, in which the hay infusion was changed every day, the animals divided a few times, then died. It was thus impossible to carry these through the fission experiment parallel with the others, in order to determine their relative rate of fission. In the case of the first two individuals tested, four series from each were carried, making eight lines in all. At first all multiplied rapidly; then at about the fifth day the rate of fission decreased; the animals showed abnormal conditions, and all of the eight lines died after seven to thirteen generations. The following table 6 shows the history of the eight lines of this race

TABLE 6

History of the eight lines of the race 43, from the beginning of the experiment till death, showing the number of fissions per day, compared with four lines of the race *L*<sub>2</sub> at the same time and under the same conditions.

		Mar. 31	Apr. 1	Apr. 2	Apr. 3	Apr. 4	Apr. 5	Apr. 6	Apr. 7	Apr. 8	Apr. 9	Apr. 10	Apr. 11
Race 43	$\left\{ \begin{array}{l} a_1 \\ a_2 \\ a_3 \\ a_4 \end{array} \right.$	2	2	1	2	1	0	0	0	(-)			
	$\left\{ \begin{array}{l} b_1 \\ b_2 \\ b_3 \\ b_4 \end{array} \right.$	1	2	2	1	1	-	(1)	(2)	(0)	(-)		
		2	1	2	2	2	1	1	-				
		2	1	2	2	2	1	1	-				
		2	1	2	2	2	1	1	-				
		2	1	2	2	2	1	1	-				
		2	1	2	2	2	1	1	-				
		2	1	2	2	2	1	1	-				
Race <i>L</i> <sub>1</sub>	$\left\{ \begin{array}{l} a_1 \\ a_2 \\ a_3 \\ a_4 \end{array} \right.$	2	1	1	2	1	1	2	2	1	1	2	1 etc.
		1	2	1	2	1	2	1	2	0	2	1	1 etc.
		1	0	0	-	(2)	(1)	(2)	(2)	(1)	(1)	(2)	(1) etc.
		2	1	2	2	1	2	1	2	1	1	1	2 etc.

(The dashes indicate death of the line. The numbers and dashes in parentheses show that the series was supplied by taking an individual from one of the surviving lines of the same race, till this also died. The lines of *L*<sub>2</sub> flourished long after the period shown, till the experiment was discontinued.)

43, together with the history for comparison, of four lines of the similar race  $L_2$ , on the same days and under identical conditions in every respect.

After all the eight lines of race 43 had thus died out, two more individuals were taken from the general culture of 43 and eight lines from them cultivated as before. These again all died out after about half a dozen fissions. A third set of eight lines, from two other individuals of race 43, had the same fate.

Thus it is clear that the race 43, though living in a large culture made from timothy hay and tap water, precisely like that in which the other races are living, cannot be cultivated in slide cultures of fresh hay infusion changed every day, though most of the other races flourish under this treatment. It is apparently the continued freshness of the infusion that destroys the individuals of race 43; if the infusion is allowed to stand for weeks, with the decaying stalks of hay, this race flourishes.

Other illustrations of the fact that the different races are adapted to different cultural conditions came to light in the experiments in which races of different size were placed together in the same culture vessel (see p. 516). Owing to the difference in size it was easy to recognize the individuals of the two races in the mixture, and in doubtful cases isolation and propagation, with measurements of the progeny, gave absolutely certain determinations. In many mixtures of this kind one of the races frequently died out, after lapse of a considerable period, while the other race continued to exist unchanged. Thus,  $k$  and  $i$  were mixed October 8, 1908; on February 25, 1909, only  $k$  was found in the mixture. Again,  $D$  and  $i$  were mixed October 21, 1908; on February 25, 1909, only  $i$  was present.  $L_2$  and  $i$  were mixed March 10, 1909; in April, 1910, only  $i$  was found in the culture. Thus it is clear that the different races are adapted to somewhat different conditions, so that one often dies out while another flourishes.

It may be noticed that if the fact had not been known that two races of diverse size were present in these mixtures, then measurements of those present would give very different results at the beginning and at the end of the culture. This might readily be attributed to the direct action of the environment, and if one of

the races had died out completely, the effects of this environmental action would appear to be permanent. Thus, study of the effects of the environment are likely to be most deceptive when made upon organisms not positively known to be pure as to race. The only safe method for such study is to begin with a single individual, from which all the specimens studied must be derived, and to take the most rigid precautions against accidental introduction of individuals of another race.

It is very probable that study of reactions to stimuli in the different races, and of other physiological matters, would show diversities among the different races in these respects. Such a study will probably be made later.

#### 6. *Relation of the races to the described species of Paramecium*

What relation have the races here described to the various named species of Paramecium, aside from caudatum and aurelia? Are possibly some of these named species based on observation of these races?

A review of the various species of Paramecium that have been described shows that in these races we have to do only with the two species *P. aurelia* and *P. caudatum*.

Ehrenberg ('38) informs us that up to his time no less than fifty-six specific names had been given under the genus Paramecium. But when we recall that under this generic name the old observers signified merely small animals that were, as Müller (1786) defines it, "inconspicuous, simple, pellucid, membranaceous, oblong," it will be understood that most of these had nothing to do with what we now understand by the genus Paramecium. Indeed, only a single one of these fifty-six, *P. aurelia* Müller, belonged to our present genus. Müller (1786) had attributed five species to the genus, under the specific names *oviferum*, *marginatum*, *aurelia*, *chrysalis*, and *versutum*; all but *aurelia* belong elsewhere.

Ehrenberg ('38) did but little better. He attributed eight species to Paramecium, under the specific names *aurelia*, *caudatum*, *chrysalis*, *colpoda*, *sinaiticum*, *ovatum*, *compressum*, *miliun*.

The first two are the only ones that belong to *Paramecium* as now understood. The third is *Pleuronema chrysalis*, the fourth *Colpidium colpoda*. The other four are so imperfectly described that they have apparently not been recognized, save that it is clear that none of them are species of *Paramecium*. Ehrenberg further described as "*Loxodes bursaria*" the animal which is now considered one of the well established species of *Paramecium* (*P. bursaria*).

Perty ('52) described *P. griseolum* and *P. aureolum*, but the descriptions are so poor that the animals can hardly be recognized. The former according to Maupas ('83, p. 451) is a species of *Cryptochilum*. The latter has apparently not been placed. Perty's *P. versutum* is *P. bursaria* Ehr., according to Claparède et Lachmann ('68, p. 36).

Claparède et Lachmann ('68) add to the list of species *P. putrinum*, *P. inversum*, *P. microstomum*, *P. glaucum* and *P. ovale*. *P. inversum*, *P. microstomum* and *P. ovale* are evidently not species of *Paramecium*, as at present understood. *P. glaucum* was based upon imperfect observation of a single marine specimen, which bore no resemblance to any form of *Paramecium* now known. *Paramecium putrinum* is still an accepted species of this genus, though perhaps with little justification, as we shall see.

Kent ('82, p. 488) describes *Paramecium marina*, a marine form with but a single contractile vacuole, in the rear. It seems not to have been seen again; in any case it evidently does not resemble the animals with which we are dealing, which all have the two contractile vacuoles. Gourret et Roeser ('86) describe *Paramecium pyriforme*, a marine form, pear-shaped, flattened dorsoventrally, broad and rounded behind, with a caudal tuft of long cilia; mouth large and open and *furnished with prominent membrane-like lips*. Bütschli ('89, p. 1711) doubts the correctness of this description and holds that the species is not a well based one, and it is not accepted by Schewiakoff ('96). In any case, it clearly has nothing to do with the animals here studied. *Paramecium trichium* was described by Stokes ('88); it is said by Schewiakoff ('96, p. 343) to be the same as *P. putrinum* Cl. et L.

There thus remain belonging to the genus *Paramecium* the species *aurelia*, *caudatum*, *bursaria*, and perhaps *putrinum*; these are the four species accepted by Schewiakoff in his monograph of the holotrichous infusoria ('96). Further, the two marine forms *P. marina* Kent and *P. pyriforme* Gour. et Roeser may possibly belong to the genus; we have already shown that they have no resemblance to the races that form the subject of this paper.

*Paramecium bursaria*, as is well known, is a short and broad form, the breadth being half or more than half the length. Accessible figures are given by Ehrenberg ('38), Kent ('82), Bütschli ('89), Schewiakoff ('96). To show the proportions, an outline of Schewiakoff's figure is copied in our fig. 24, *a*. This species is said as a rule to have rounded granules in the inner layer of the ectosarc, and these, together with the endosarc, usually contain green zoöchlorellae, giving the animal a green color. Trichocysts are generally present. But the rounded granules, the zoöchlorellae and the trichocysts may be lacking in some individuals. The anus is at the posterior end.

*Paramecium putrinum* Cl. and L. is the same as *P. bursaria*, save that it lacks the granules, zoöchlorellae and trichocysts, and has the anus on the ventral surface near the posterior end instead of at the posterior end. As all these characteristics save the last likewise occur in *P. bursaria*, the position of the anus remains the only distinguishing feature. This slight variation in a most undefined character would hardly appear to furnish grounds for a specific distinction, as these distinctions are commonly made, and as a matter of fact we find that Claparède and Lachmann described this as a separate species on entirely different grounds. They say that they would not have attempted to distinguish this as a separate species, save for the fact that its *embryos* are very different from those of *P. bursaria* ('68, p. 266). Now these supposed embryos, as is well known, were really parasitic Suctoria, so that they have nothing to do with the specific characters of the parasitized animals; it was doubtless accident that the two sets of infusoria observed by Claparède and Lachmann happened to have different parasites. It is owing to this accident that the two species have been established.

However, from the varying descriptions as to trichocysts, etc., it is probable that there exist a number of diverse races of *P. bursaria*, just as there do of *P. caudatum* and *P. aurelia*; some of these then might be called *P. putrinum*. An outline copy of Schewiakoff's figure of the latter is given in our fig. 24, *b*, to show the form. A figure of *P. putrinum*, copied from Roux ('99) is given in Lang's *Lehrbuch* ('01). Roux represents *P. putrinum* as having a tuft of longer cilia at the posterior end, while according to Schewiakoff ('96) it is without such a tuft.

It is clear from the form and other characteristics that *P. bursaria* and *P. putrinum* are not the animals we are dealing with in the present paper.<sup>11</sup> All in all, it is evident that none of the named species of *Paramecium* are based upon the races described in this paper, save *P. aurelia* and *P. caudatum*. When any of the races were observed, they have been referred to one or the other of these two species.

#### 7. *Indications of the existence of diverse races of Protozoa in the reports of other observers*

It appears probable that many of the diversities in the descriptions of the various species of *Paramecium*, given by different observers, are due to the fact that different races were under observation. The reported differences in size are doubtless partly due to different environmental conditions<sup>12</sup>, but it is clear that we must not go too far in attributing all size differences to this. The differences due to diversity of race are much more extensive, besides being permanent and common.

<sup>11</sup> Only when individuals of *caudatum* or *aurelia* are excessively fed do they take on an oval form in any way similar to that of *bursaria* or *putrinum* (See fig. 22). But under such conditions the peristomal groove practically disappears, whereas it is represented as evident in the species last named. Furthermore, *caudatum* and *aurelia* are almost never seen in this condition in the wild state, and the slender form reappears as soon as food becomes scarce. The species *bursaria* and *putrinum* have been observed for long periods by many observers, so that it is clear that they are not mere temporary forms of *caudatum* or *aurelia*.

<sup>12</sup> See Jennings ('08) for an exhaustive study of the effects of varied environment in causing differences in size in *P. caudatum* and *P. aurelia*.



It would not be difficult to collect from the literature observations indicating the existence in Paramecium and other infusoria of races differing in inherited characteristics. • One or two examples of this must suffice. Maupas, in his paper of 1888, says that all the progeny of a single individual are alike morphologically and physiologically (p. 176). Differences in rate of fission, etc., do show themselves among different individuals of the same *species*, but Maupas is convinced that in such cases we are dealing with progeny of different original parents; that they belong, in modern terms, to different "pure lines" with different racial characteristics (pp. 203–204). In *Onychodromus* he finds hereditary differences in the rate of fission, in different lines (pp. 220–221). The same thing is observed in different lines of *Leucophrys* (pp. 241–242).

Gruber ('92) described "dwarfs" of *Stentor coeruleus* and *Stentor polymorphus* (I have not been able to see the original paper).

Enriques, after an extensive physiological study of "*Colpoda steini*," discovered that this consists of two sets of individuals, with permanent inherited differences of size ('08, p. 272). One set consists of small individuals, which may readily be induced to conjugate; the other of larger specimens, that do not readily conjugate. On account of these differentiations in size and in physiology, Enriques considers these to be distinct species. To the larger race he gives the name *Colpoda maupasi*, while the smaller retains the name *Colpoda steini*. (For full account of these, see Enriques, '08a). It is to be noticed that the differences between these two species are of the same character as the differences between two of our races of Paramecium of the aurelia group. The race *k* differs from the race *i* in size and in greater readiness to conjugate. We might therefore distinguish these as two different species. The chief objection to this is that the number of such races is in Paramecium so great, and the differences between them often so slight, that the giving of separate specific names to each would confuse rather than clear the matter. There is the further possibility that the different aurelia races may be found to interconjugate, giving intermediate forms; this has not yet been observed.



Popoff in his recent brilliant studies on various infusoria finds frequent indications of the existence of diverse races of the same species. Thus, in *Frontonia*, if a culture is started from a single individual, the size is found to be more uniform than if started from a number of individuals ('08, p. 269). This indicates of course that among the original individuals there were hereditary differences in size. In *Stylonychia* he finds that different cultures, each derived from a single individual, show characteristic differences in mean size, and attributes this to hereditary differences in the sizes of the original specimens from which the cultures came ('08, p. 345). In the second of his "Zellstudien" ('09), Popoff gives a number of examples of inherited differences in size in *Stentor* and *Frontonia*, together with what he believes to be the explanation of the origin of the differences. This explanation we shall take up later. Of special interest is the indication of the existence of different races of *Paramecium*, mentioned in the postscript of Popoff's paper ('09, p. 180). Study of the ratio of the volume of the cytoplasm to the volume of the nucleus carried out on *Paramecium* by three different investigators in the same laboratory gave ratios in the different cases of 15 to 1, 30 to 1 and 45 to 1. These great differences naturally suggest that different races of *Paramecium* were under consideration, and an investigation of this possibility is promised us, by Rautmann.

It is desirable that an investigation be made of the ratio of nucleus to cytoplasm in the races described in the present paper; this has not yet been done.

McClendon ('09) observed in *Paramecium* the existence of a number of races of diverse size, the relative sizes remaining the same for long periods and under varying conditions of culture. One of these had but one micro-nucleus, belonging thus to the caudatum group, while two others had two micro-nuclei, and therefore belong to the aurelia group. The largest aurelia race observed by McClendon was as large as the caudatum race, a result never reached with the races cultivated in this laboratory.

8. *Diverse, closely related races in other organisms*

It is well known that in more complex organisms belonging to a single species many races exist, differing very slightly in hereditary characters. The extensive work upon this matter has come to be known as "pure line" work, following the example of Johannsen ('03), who showed that many slightly differing "pure lines" exist in beans and barley when self fertilized. The work of de Vries, Nilssen, Shull, East, and many others, have shown the existence in higher plants of many diverse strains, differing slightly but permanently in hereditary characters. Johannsen ('09) has recently proposed as a designation for such diverse races or strains the word *genotype*, and this usage has been followed by others. Mr. T. D. A. Cockerell has called my attention to the fact that the word *genotype* is already in use with another signification, namely, that of designating "any typical material of the type species of a genus." It may be necessary therefore to replace Johannsen's term *genotype* by some other, as a name for the diverse strains of which a species is formed.

The question of the existence of diverse permanently differentiated strains among lower animals is one of much interest from many points of view, and it has yet been comparatively little studied. The existence of many diverse forms grouped under a single specific name is well known, particularly in plankton organisms, where this has been the object of a recent monumental work by Wesenberg-Lund ('08). This author shows the existence of great numbers of diverse forms of Algae, Protozoa, Crustacea, Rotifers, etc., at different seasons of the year or in different localities—each "species" including many such forms. But these are commonly spoken of as "seasonal variations" and "local variations," and there is a tendency toward the view that these are all modifications due to environmental conditions of what is essentially a single but very plastic stock. According to this view, the same "pure line" would take on one or another of these forms, depending on the conditions under which it existed. The usual opinion is well put by Wesenberg-Lund as follows: "It is only since '95 that the view has become more and more general,

that the variations in form could not be used as differentiating marks of species or varieties, but were called forth by variations in the surrounding medium and changed not only from place to place, but also from time to time."

Of course another view is possible; that the different forms really belong to different lines or strains, one strain replacing another by hatching out of the eggs as the environment favorable for development of this strain appears. These two views are discussed by Wesenberg-Lund; he points out that evidence for deciding between them is in most cases quite lacking. Which view is correct can only be determined by breeding experiments with carefully isolated lines. The work of Wesenberg-Lund furnishes a vast mine of suggestions for most interesting experimental work. Breeding experiments with pure lines should be carried on with such polymorphic forms as *Anuraea cochlearis* and *Brachionus bakeri* among the rotifers; with the *Daphnias*, *Cyclops*, *Ostracoda*, among the Crustacea, etc. Different lines should be subjected to the same environment, to see if they become identical; different parts of the same line to different environments, to see how far they take on different forms. The work thus far done with pure lines suggests the probability that the differing forms will be found much more constant than had been supposed, many permanently differentiated races existing, though differing environments will cause variations of form in each race, but in a rather limited degree. This appears to be the view to which Wesenberg-Lund himself has gradually come, though he feels the need of experimental evidence. One or two investigations along this line on such lower animals should be mentioned. Hanel ('07) reported that many different lines of *Hydra grisea* exist, characterized by having different average numbers of tentacles, though the number within each line varies with environmental conditions. Hase ('09) however has recently denied that the number of tentacles is hereditary at all, thus doing away with the existence of these diverse lines.

Woltereck ('08) examined two local races of *Daphnia longispina* and found that the differences were permanent and hereditary, even when the races were cultivated for two years under the

same conditions. A less extended examination of a number of other local forms of *Daphnia* showed these likewise to be permanent. By diverse alterations in the environmental conditions it was easy to modify the various races in such a way as to cause their characteristics to overlap, but resemblances so caused were not hereditary, in the sense of being retained when the two races were bred under the same conditions. These results of Woltereck with *Daphnia* are thus quite parallel with our own for *Paramecium*; it is probable that they are typical of what will generally be found in organisms.

These minutely differing "races," "pure lines" or "genotypes" in lower organisms evidently correspond to "hereditary individual differences" in such an organism as man. Thus every individual of man probably represents a different "genotype" from every other, save in the case of identical twins, which apparently belong to the same genotype. The application of the genotype concept to man will best be realized by conceiving sexual reproduction to cease, and each individual to reproduce by budding or fission, as in *Hydra* or *Paramecium*. We should then have as many diverse pure lines as there are individuals with diverse hereditary characters. Thus there is no other organism in which we have so extensive and minute a knowledge of "genotypic" differentiation as in man.

### 9. *Origin of the diverse races*

It is to the origin of the diverse races that further investigations will be directed; the present paper does not deal primarily with this point. But it is needful to bring out certain points, particularly in regard to some theories that have been proposed on this matter.

Popoff in his recent series of brilliant papers ('08-'09) has set forth an explanation of how races of different size arise, particularly in the Protozoa, though the same process applies less directly also to the Metazoa. Popoff's view is, in a word, that cells of different size arise as a result of inequalities in nuclear division. There is conceived to be a definite proportionality, under given

conditions, between the volume of the nucleus and the volume of cytoplasm; hence between the size of the nucleus and the size of the cell. The amount of cytoplasmic material is readily regulated so as to correspond with the volume of the nucleus. Hence if at a given fission, as of *Paramecium* or *Stentor*, the nucleus divides unequally, the cells taking origin at that time will likewise be unequal. If now in future fissions both the cells divide as a rule equally, we shall have two races permanently differentiated in size. The explanation holds that in most fissions nuclear divisions are equal, resulting in inheritance of a given typical size in all the cells produced, but that occasionally, through accident or otherwise, an unequal nuclear division occurs, giving rise to races of different size. The same thing is conceived to occur in the formation of germ cells in Metazoa, the bodies formed of the smaller cells being thus smaller—so that small races of both Metazoa and Protozoa arise in this way.

Popoff first expounds this view on theoretical grounds, based on general considerations following from Hertwig's theory of the "Kernplasmarelation," taken in connection with the observed existence of individuals of different size (Popoff, '08, p. 269, 274-275; 344-351). In a later paper ('09) he supports it by observations and experiments, coming finally to the conclusion that "these results . . . . show clearly that the cell-size in a Protozoan species is not something definite, but that it is extremely variable, easily fixed at any desired grade of size, and transmissible from generation to generation" (p. 148). There is a certain amount of contrast between the condition that one would expect to find if the cell size is "not something definite," but "extremely variable," and the results of my study on precisely this point in *Paramecium*, in my paper of 1908. In this study the cell size was not found to be variable within a pure line, but on the contrary showed a remarkable constancy, so that it was not possible by long continued selection of large specimens or small specimens from a given race to produce a race differing in size. These results of course do not show that change in size may not sometimes occur in the manner set forth by Popoff. But the investigation in *Paramecium* is felt to have been sufficiently extensive and thor-

ough to indicate that such changes do not occur readily nor often in that animal, and to make desirable a very careful examination of the evidence submitted by Popoff, in order to see how far it is demonstrative.

In *Paramecium* it is very easy to find races of differing size when one is working with a culture not known to be derived from a single individual, but this changes completely as soon as our culture is known thus to have origin from a single specimen. If one assumed, to begin with, that the *Paramecia* in a "wild" culture were all alike, it would be easy to collect evidence seeming to indicate that differentiation in size had occurred; but the conclusion so drawn would be an entirely mistaken one. Demonstrative evidence of the origin of heritable differences in size can then come only when it is known positively that the differing individuals or lines were derived originally from a single specimen. This requirement can be met in two ways: (1) by working with a culture that was begun with a single individual and has been kept uncontaminated; (2) by actual observation of the fission that produced the two lines of different sizes—with of course immediate isolation of the two products of the fission.

How far do the cases set forth by Popoff meet these requirements? In his summing up Popoff says, "In the discussion up to this point I have set forth nine cases in *Stentor coeruleus* (including the specimens of normal size) and two in *Frontonia leucas*, in which it was possible by inequalities of division or by experimental operation to change and to fix the cell size, and to cultivate the size-varieties thus produced for a long time side by side" ('09, p. 163). It will be worth while for us to look into each of these cases, to see how far they fulfil the conditions required for demonstration. These conditions, as we have seen, are:

1. Either the entire culture from which the new race comes shall be known to have been derived from a single specimen;
2. Or, the author shall have observed the fission that produced the unequal races, isolating the two individuals produced at once, so as to know that both came from the same individual.

Now, it should first be noted that since Popoff (as we have seen) counts in his eleven cases the unchanged normal specimens

as well as the new races produced—if we ask the question: In how many cases does he claim to have seen new races produced?—the number falls at once to at most *six*. Let us examine these.

1. The first case ('09, p. 144-145) relates to Stentor. Here condition no. 1 was not fulfilled, since the author nowhere states that the culture was derived from a single individual. As to condition no. 2, at first reading one gets the impression that this was fulfilled, but a more careful study shows that it was not. The facts are set forth as follows: "In a Stentor culture that I had set in progress for various experiments and that I looked through carefully everyday, there appeared from time to time, although seldom, a few Stentors, which were at once noticeable on account of their smaller size. . . . All these small Stentors were the product of chance inequalities in fission" (p. 144). This latter statement would seem to cover our second requirement, till we inquire as to the basis on which it is made. The author states "These irregular divisions I was able to observe *in a few cases*" ("ein paarmal") (p. 145). The statement that *all* were due to such unequal divisions is thus an assumption; the question of importance is whether he observed the unequal division and isolated its products in the particular case where he believes that two diverse races resulted. On this point again the first general impression is that he did, for he says, "On November 11, 1907, I separated from the main Stentor culture a small animal that had arisen through unequal fission" (p. 145)—but again careful examination of the text shows that he did not observe this unequal fission but merely assumes it, for he gives an extract from his note-book as to the first appearance of this small specimen, where fission is not mentioned at all. It is simply this: "11. XI. 07. A small Stentor coeruleus. Nucleo-plasmar relation normal." Now this small individual was cultivated separately, and its progeny retained the small size. But since the original culture is not stated to have come from one individual and since the author does not say that he observed the fission by which the small specimen arose, and since he did not isolate and cultivate the other product of the supposed unequal fission to see if it was larger—it is possible, indeed probable, that the original culture contained



racés of various sizes, and that what the author did was to isolate a representative of a small race. Certainly this would be the explanation in the case of Paramecium, and in the account as Popoff gives it there is absolutely no proof that the small race was derived from one of different size.

2. The second case is that of a Stentor from the same culture but somewhat above the usual size; this when isolated gave progeny above the usual size (p. 145-147). Here again we have a culture which is not said to have come from one individual; the author does not know the size of the ancestors of his large race; he did not isolate the two products of the supposed unequal fission and observe that they gave races of unequal size. There is thus no proof whatever that the large race had arisen from one of a different size. If the original culture, like a wild culture of Paramecium, contained races of varying size, this gives a full explanation of all the facts as Popoff states them.

3. The third case is in *Frontonia leucas* (p. 147-148). Here the facts are in almost every detail parallel with those of cases 1 and 2 in Stentor. The original culture is not said to have been derived from a single individual. On November 11, large and small specimens were isolated, but these are not said to have come from the fission of a single individual. Their progeny retained the relative sizes of the parents, showing that they belonged to different races. The facts can be readily paralleled in any "wild" culture of Paramecium—but the explanation is that diverse races were present in the culture from the beginning.

(Popoff in the text speaks of the small specimen as "Eine durch ungleichmässige Teilung entstandene, auffallend kleine *Frontonia*" (p. 147), but again his extract from his note-book on the same page, indicates clearly that the idea that this specimen came from unequal division was an assumption, based on his general theory. The note-book says merely "11. XI. 07. Die Kultur mit einem sehr kleinen Tier angelegt." So important a point as the exact observation of its origin would surely not have been omitted. In any case, the author had no knowledge of the normal size of the line of individuals from which this small specimen came.)



4. The fourth case (p. 149) relates to the isolation of a small Stentor, whose progeny retained the small size. Again the original culture is not stated to have been derived from a single individual, so that it may have contained races of different size. Again, the author does not know the characteristics of the line of ancestors from which the small specimen came. There is thus no evidence that the small race arose from one of a different size.

The two remaining cases (5 and 6) are based on experimental operations; they give better evidence than those we have considered.

5. The fifth case (p. 153-154) was as follows: By centrifuging a Stentor for five hours, it was caused to divide unequally, the smaller part receiving three nodes of the nucleus, the larger sixteen. This was on April 9. On April 11 both parts had grown a new peristome. "From this moment on, fission took place regularly. Up to April 18, on which day the cultures came to an end on account of unforeseen lack of food, the size at fission showed no change. In this way two size-varieties of Stentor were produced, which retained further their (relative) sizes" (p. 154).

This experiment would be demonstrative, save for the fact that the cultures were kept only seven days after the normal form was restored. In an earlier paper ('08a) I showed that in *Paramecium* abnormal bodily conditions are often handed on for a number of generations, but that as a rule they are finally gotten rid of, and the normal condition is restored. It is entirely possible that this would have occurred in the case of the abnormal size of Stentor. Popoff does not tell us how many fissions took place in the seven days; as the animals had recently been operated on, fission was probably slow. It is not at all surprising that the abnormal condition had not been fully regulated in this brief period even though it might have been regulated later. The experiment is not therefore a demonstrative one.

6. The sixth and last case (p. 157-262) relates to a Stentor which was isolated from a "room culture"; by subjecting this animal to cold a fission that had begun was suppressed. Various abnormalities and monstrosities resulted, but there finally appeared from it a race of Stentors much larger than usual, with

larger nuclei. The culture of large individuals was kept fifty-three days.

The vulnerable point in this case is the fact that the original "room culture" is not stated to have come from a single individual. It may therefore have contained various races; and it is possible that what the author did was merely to isolate an individual of one of the large races—the suppressed fission having nothing to do with the greater size. This would fully explain the great size in the isolated culture, as compared with the usual size in the room culture. According to Popoff, there were a number of unequal fissions in this large race, producing small individuals. Here was a chance for a crucial test of the question whether from a race of a given size another of different size is produced. If these small specimens had been cultivated separately, and had given a small race, while the original race continued large, all conditions for a demonstration would have been fulfilled. But unfortunately, the author merely removed these small specimens, in order that they might not bring down the average size of the parent culture; we do not know what they would have produced if farther cultivated.

In making this analysis there is no desire to detract from the value and interest of Popoff's beautiful work. He has put forward almost the only plausible suggestion thus far as to the origin of the races of different size, and the cases numbered here 5 and 6 may be said to furnish a certain amount of evidence for the correctness of this view. If the suggestion does turn out the correct one, it will be of extraordinary interest. But we are here confronted with a precise and definite question: Has it been demonstrated correct or has it not? I am sure that Popoff will agree with us that a cardinal principle for the advance of experimental science is that nothing shall be held demonstrated that has not been demonstrated. If it is true that the heritable cell size is "not something definite, but is extremely variable," then there should be no difficulty in getting cases fulfilling the simple and definite conditions that will furnish a demonstration. In my work with Paramecium I have found it easy to get races of diverse sizes so long as these precise conditions are not fulfilled;

but as soon as they are fulfilled, the heritable size remains constant. It was therefore important to determine whether these conditions had been fulfilled in the cases cited by Popoff. Analysis seems to show that they had not. If in this analysis any essential points have been overlooked, Popoff will be able to set the matter right.

This question should be readily resolvable by experiments in cutting *Stentor* or *Spirostomum* into pieces. Lillie ('96) showed that pieces of *Stentor* one-twenty-seventh the entire animal will regenerate. It should not be difficult to get such small pieces containing a proportionately small fragment of the nucleus and to determine by culture whether the race produced by such a small specimen remains small, or whether it finally returns to the normal size. Popoff's work shows that the volume of the cytoplasm can be regulated to accommodate itself to the size of the nucleus. His conception is that new races are produced when both nucleus and cytoplasm are smaller (or larger) than usual; since it is the *proportionality* that is restored, not the absolute size. This can be subjected to rigid experimental test by such work as we have mentioned.

In any case, of course, the method of action set forth by Popoff, even if it turns out to be correct, would account only for the differences in size. The physiological differences set forth in this paper, and the differences in form, in structure of micro-nuclei, and the like—the origin of these requires other methods of action. The problem will be dealt with in the further investigations on *Paramecium*.

10. *List of the races or lines dealt with in this paper, with their characteristics*

In conclusion it will be well to give here in brief, for reference, a tabulation of the races or lines mentioned in this paper; this will further serve as a sort of summary indicating the sort of racial diversities one is likely to meet in working with Protozoa. Each race or line is derived from a single individual. The designations used are the same as those employed by Jennings in 1908, and in the present paper.

1. *Caudatum Group* (*Paramecium caudatum* Ehr.). One micronucleus, of the structure shown in fig. 8. Animals larger, slightly more slender, posterior half of the body tapering more rapidly and regularly than in the aurelia group.

$L_2$ . The largest race that was thoroughly studied; mean length under different conditions, 180 to 230 microns. Two years and four months in the laboratory. (See Estabrook, '10, for an extensive study of growth in this race.)

$A_1$ . Similar to  $L_2$ , but a little smaller.

$G_1$ . Similar to  $A_1$ .

20. Similar to  $L_2$ ; conditions inducing fission differing from those for  $L_2$  (see p. 514).

43. A large race, differing physiologically from others. Precise size relative to other races not determinable, because it does not live in the pure hay infusion employed as a culture medium.

$D$ . Smallest caudatum race studied; mean length about 175 microns under conditions in which  $L_2$  has a length of 200 microns or more. More than three years in the laboratory. (See Jennings, '08, for extensive studies of growth and environmental action in this race.)

2. *Aurelia Group* (*Paramecium aurelia* Müller). Two micronuclei, of the structure shown in fig. 9. Animals smaller, slightly broader in proportion to the length, and tapering less rapidly from the middle backward, than in the caudatum group.

$k$ . One of the larger aurelia races; length about 125 microns, under conditions where  $L_2$  has a length of about 200 microns. Conjugates frequently and readily. Rate of fission same as in the caudatum group. Two years and four months in the laboratory. (See Jennings, '10, for a study of the conditions inducing conjugation, based largely on this race.)

$C_2$ . Size about the same as in  $k$ ; rate of fission slower than in  $k$ . Two years and four months in the laboratory.

$g$ . Size about as in  $k$  and  $C_2$ . One year and six months in the laboratory. (See Jennings, '08, for experiments on selection in this race.)

$c$ . Smaller than the races thus far mentioned; length about 100 microns, under conditions in which the length of  $k$  is about 125

microns. Conjugates much less readily than *k*. Rate of fission greater than in *k*. More than three years in the laboratory. (See Jennings, '08, for extensive studies of growth, environmental action and selection in this race.)

*i*. The smallest race studied; length about 90 microns, under conditions in which *c* has a length of about 100 microns. Conjugates less readily than *k*. Rate of fission as in *c*, more rapid than in *k*. Two years and six months in the laboratory. (See Jennings, '08, for many measurements of *i*, in comparison with *g*, and for studies of the effect of selection in this race.)

A word may here be added regarding the distribution of the various races. There appears to be a general impression that animals answering to the description of aurelia are rare as compared with caudatum; Calkins ('06) urged this as one argument against the distinctness of *P. aurelia*. My experience has not confirmed this impression. The small forms belonging to *P. aurelia* are not readily visible to the naked eye, and are not conspicuous even with the microscope, while the large individuals of *P. caudatum* are very conspicuous; it is natural, therefore, that the former do not attract attention so readily as the latter. I believe that this is the reason why caudatum has been supposed more common. My impression from long continued work with the diverse races is that aurelia is as frequent as caudatum. Many samples of "wild" material, from ponds, drains, etc., contain races of both aurelia and caudatum. Others contain aurelia only; others caudatum only. Apparently samples containing aurelia occur as often as those containing caudatum.

#### 11. *Precautions necessary for work with pure races*

A final word should be said regarding methods of work in studying these races. Demonstration of the constancy and uniformity of the races can of course come only when the most rigid care is taken to prevent contamination and admixture. By accidental transference of individuals of one race to cultures of another (which may happen with the greatest ease), it will of course be easy to get striking cases of apparent splitting of one

race into two, or transformation of one race into another. The fact that no cases of this have occurred in my three years' work where many races existed side by side in the same laboratory, must weigh heavily in interpreting any cases in which one race apparently gives rise to another.

The precautions required against admixture are mainly those for preventing fluid containing individuals of one race from getting into a culture containing another. Pipettes used in handling the animals should invariably be thoroughly rinsed in hot water immediately after they are used; for this purpose it is necessary to have at hand at all times during work a vessel of water over the gas flame. Neglect of this precaution is fatal. All fluid added to the cultures should be heated beforehand to at least 50 degrees Centigrade to destroy any Paramecia it contains. It is important not to make the mistake of supposing that Paramecia can be filtered out by the use of filter paper; a trial of this on a rich culture of Paramecium, with subsequent microscopic examination of the fluid which comes through will convince anyone of the mistake of this procedure.

On the other hand, my experience does not indicate that there is any danger of transferring the animals dry, either in the air, as dust, or attached to solids. Dry hay added to water containing no Paramecia never gives a culture of Paramecium. Adding dry hay to a culture of a single race never results in an admixture of races. Leaving open a culture vessel containing a single uniform race, even in a laboratory containing dozens of cultures of other races, never results in an admixture of races, provided that no fluid is allowed to drop into the open culture. On all these points my experience has been extensive and long continued, with absolutely concordant results. There is no experimental evidence from any source that Paramecium may be dried, and later revive when introduced into water.

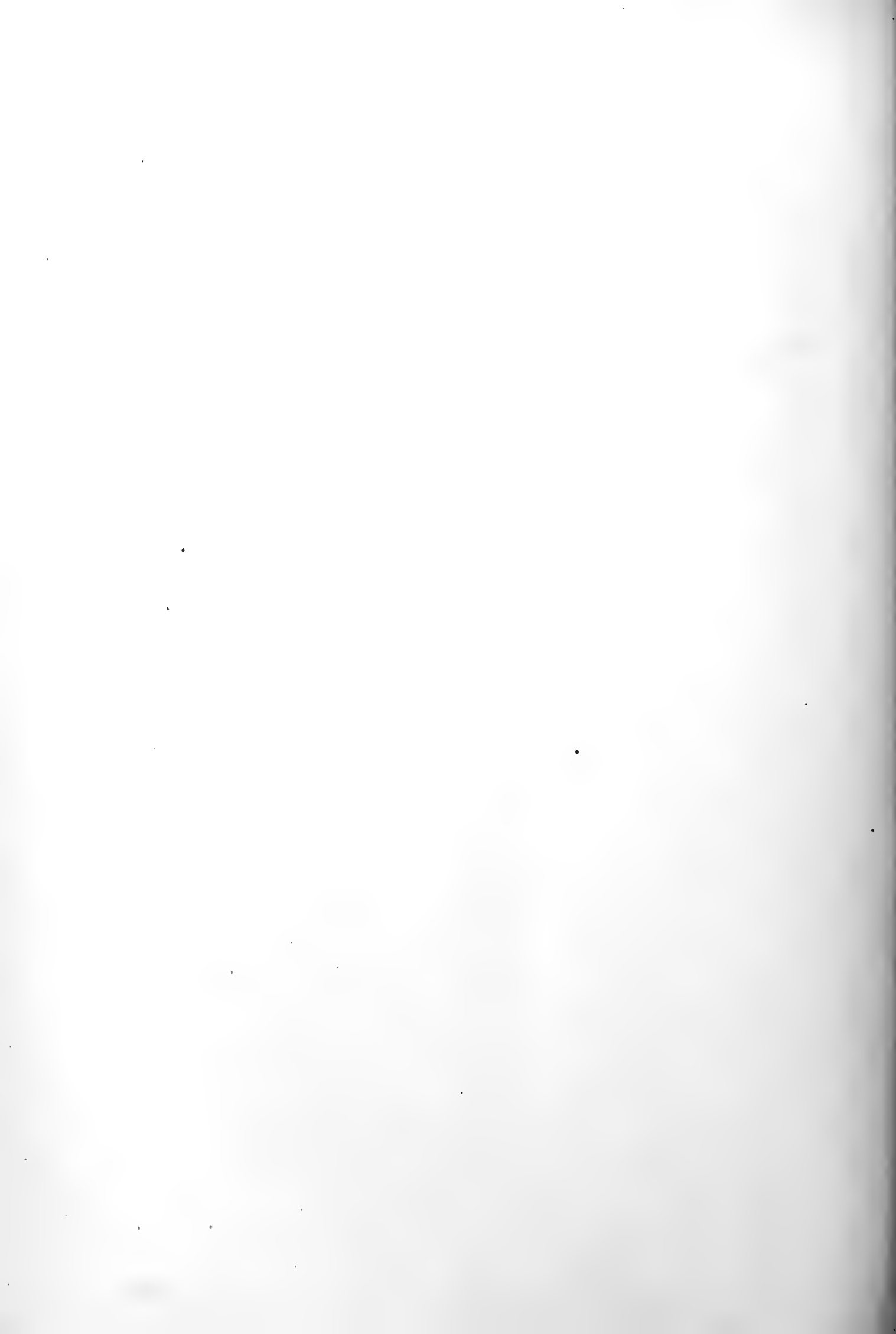
Thus there is great danger in the introduction of fluids into the culture; little or none in the addition of dry substances.

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# SEGREGATION OF THE GERM-CELLS OF THE TELEOST, LOPHIUS<sup>1</sup>

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THIRTY-FOUR FIGURES

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<sup>1</sup> Thesis presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

## INTRODUCTION

The study of the segregation of the germ-cells is of interest from two points of view; first, in relation to the general study of problems of heredity; and, second as a part of the problem of embryonic differentiation.

The mechanism by which the qualities of one generation become repeated in the next has long been a subject for speculation and investigation. The more recent views of the separateness of germ plasm from somatic structures receive their verification or refutation from studies of the actual separation of the germ-cells and body cells in the early developmental stages of the individual. The segregation of the reproductive cells in early development would establish a continuity between the germ-cells of succeeding generations without the intervention of the specialized tissues of the animal body.

In relation to the mechanism of cell differentiation the phenomenon is of further interest. In the history of both the race and the individual, the separation of the reproductive from the vegetative cells may be the earliest differentiation. It is this setting aside of generalized cells to transmit the characters of the race that allows the remaining cells, when relieved of this responsibility, to undergo the high degree of differentiation found in the body of the higher animal and plant. The problem of the differentiation of the germ-cells is not different from that of cell specialization in general; it is but the beginning of the longer, more general process and as such may be conditioned by the same factors. An understanding of the differentiation of the germ-cells is the first step toward a comprehension of the general embryonic differentiation that follows.

The present investigations on *Lophius piscatorius* have to do chiefly with the earlier stages and were carried on at the zoölogical laboratory of the University of Pennsylvania, and in part at the Marine Biological Laboratory, Woods Hole, Mass. The problem was suggested by Prof. Thomas H. Montgomery, Jr., to whom I am indebted for many helpful and stimulating suggestions. For certain preserved material I am further obligated to

Prof. J. S. Kingsley, of Tufts College and to Prof. H. V. Wilson of the University of North Carolina.

## I. REVIEW OF THE LITERATURE

### 1. *The Origin of the Germ-cells*

A study of the literature shows that there are three quite distinct views about the origin of the germ-cells of vertebrates, viz., (a) theory of the germinal epithelium, (b) the gonotome theory, (c) the theory of early segregation. There have also been a few writers who have held that the germ-cells arise by a combination of two of these processes.

*a. Germinal epithelium.* This view is due to Waldeyer ('70). In the embryos of various vertebrates, he first recognized the germ-cells as large rounded cells in the cylindrical epithelium covering the genital ridge. He considered that the cells arise by the transformation of the cells, among which they are observed. This region of the peritoneum has since been known as the 'germinal epithelium.' To the rounded cells observed here, he gave the name 'Ureier' (primordial ova) because he believed that the ova, but not the spermatozoa came from them. These latter he considered had their origin from the epithelium of the Wolffian duct. Later ('03) he had come to recognize that the spermatozoa, as well as the ova arose from the 'Ureier' so that he spoke also of 'Ursamenzellen.'

This view of the origin of the germ-cells was at once accepted by embryologists, and by far the greater number of writers from that time to the present have held this view. This explanation so readily fell in line with the observations, that its accuracy was taken for granted by most writers without careful research to test it rigidly.

So many have been the papers describing such an origin, or assuming it, and the subject has been so often reviewed that it is unnecessary to give special notice to those papers. Many of the more important ones are listed in the Bibliography.

b. *Gonotome theory.* The advocates of this view agree with those who hold a peritoneal origin, that the primordial ova are actually in the line of the functional sex cells. They contend however that they do not arise by the transformation of peritoneal cells, nor indeed in the peritoneum at all, but from a definite part of the segmental mesoblast of the embryo, whence they are brought into the peritoneum during the growth of the embryo. This view was first advocated by Rückert ('88) in a study of *Pristiurus*, and was given fuller expression by Van Wijhe ('89) in studies in *Scyllium* and *Pristiurus*. The later writer applied the term 'gonotome' to the part of the segment producing the sex cells. He considered that segmentation extends laterally a short distance into the lateral plate, and to this part of it he applied the term 'hypomere.' The hypomere is equivalent to the gonotome. Hall ('04) applied this theory to *Amblystoma*, and Dustin ('07) to three other Amphibia; *Triton alpestris*, *Rana fusca*, and *Bufo vulgaris*. He also describes a transformation of peritoneal cells at a later developmental period and makes these two lines of germ-cells the basis of interesting phylogenetic speculation.

c. *Early segregation.* The advocates of this view agree with the two views just presented, that the primordial ova are true germ-cells but consider that neither of the preceding explanations of their origin are correct. They hold that the germ-cells are early segregated, during cleavage stages, before organs or germ-layers are formed, and later migrate into the coelomic epithelium where they are readily recognized. According to this view they are not transformation products, nor do they arise from or belong to any germ layer, but they are primitive cells early set apart. Careful investigations upon vertebrates of every group except mammalia have shown that the germ-cells may be definitely recognized in very early stages of embryonic development, and that during the course of embryo formation they migrate into the position of the gonads. The following list gives the writers who have found evidence of an early segregation of the germ-cells of vertebrates:

Cyclostomata:	Amphibia:
Wheeler, 1900, lamprey	Nussbaum, 1880, <i>Rana fusca</i>
	• Allen, 1907a, <i>Rana pipiens</i>
Elasmobranchia:	King, 1908, <i>Bufo lentiginosus</i>
Balfour, 1876, <i>Scyllium</i>	• Kuschakewitsch, 1908, <i>Rana esculenta</i>
Beard, 1900, 1902 a, <i>Raja batis</i> , <i>Pristiurus</i>	Reptilia;
Woods, 1902, <i>Squalus acanthias</i>	• Allen, 1906, 1907 b, <i>Chrysemys marginata</i>
	• Jarvis, 1908, <i>Phrynosoma cornutum</i>
Teleostomi:	Aves:
Nussbaum, 1880, trout	Hoffmann, 1893, twelve species of birds
Jungersen, 1889, <i>Zoarces viviparus</i>	Nussbaum, 1901, chick
Eigenmann, 1891, 1896, <i>Cymatogaster aggregatus</i>	
Böhi, 1904, trout and salmon	
Federow, 1907, <i>Salmo fario</i>	
• Allen, 1909, <i>Amia</i> , <i>Lepidosteus</i>	

A study of the literature shows clearly that there still prevails considerable diversity of opinion about the origin of the cells which afterward become the functional sex-cells. In closely related species, and in some cases in the same species, different observers have reached surprisingly different conclusions. It may be of interest to review briefly the evidence and see wherein the views differ, what they have in common, and so far as possible to reconcile the conflicting opinions.

The common meeting point is seen at once to be the primordial ova recognized by Waldeyer in the germinal epithelium, forty years ago. Practically all investigators are agreed as to the nature of these cells and their later history. The differences of opinion arise in an attempt to explain their origin. Transformation is the keynote of the germinal epithelium theory and only by actual demonstration of the transformation of peritoneal cells into germ-cells can the theory be established. The literature shows that few writers have actually attempted to show evidence of such a transformation, but have merely assumed that because the earliest observed germ-cells lie among peritoneal cells they arise from them.

It further appears that of the few who have tried to show such evidence, not a single one has given a good detailed account of the

process. Moreover it is to be noted that several recent investigators who have made careful search, have been entirely unable to find any indication that there is such a transformation and emphatically state that germ-cells are not produced in this way.

Probably the circumstance which contributed much to the belief in a germinal epithelium was the inability of investigators to recognize the germ-cells in earlier stages. This failure of many observers is not to be wondered at, because in the early stages the germ-cells are very obscure, and become easily found and recognized only when they reach the peritoneum in the position of the genital ridge.

Of recent years, however, many investigators of vertebrate embryos have been able actually to show that the peritoneum is not the place of origin of the primordial ova and have clearly traced them in the earlier stages, while they are migrating to the peritoneum covering the genital ridge. Such clear positive evidence of an early origin renders worthless the doubtful evidence for a peritoneal origin. In every group of vertebrates, except the mammals where the problem is more difficult, the germ-cells have been clearly recognized before they have reached the genital ridge and in several forms they have been traced back to the beginning of embryo formation.

With regard to the gonotome theory there seems little to be said. It was never widely accepted and all the recent evidence is against it. The conditions observed by Rückert and Van Wijhe are capable of other interpretation and would probably be otherwise explained by those men themselves in the light of more recent discoveries. One recent paper of importance describing a segmental origin of the germ-cells is that of Dustin ('07) on Amphibia. The observations recorded in this paper are so directly contrary to the accounts of other recent works on this group that we are not justified in accepting his view without further evidence.

The above views of the origin of the germ-cells in vertebrates are mutually exclusive. The tracing of the germ-cells to an origin in primitive tissue of the early embryo, disproves both views

which affirm a mesoblastic origin at a later time. The sum total of evidence at the present gives reasonable grounds to believe that the germ-cells of vertebrates arise only as primitive cells set apart during cleavage stages.

## 2. *Migration of Germ-cells*

*a. Means of migration.* As it has become apparent that the germ-cells have their origin in parts of the embryo more or less remote from the location of the future germ-glands, it has become equally apparent that they undergo a corresponding change of position. There is quite general agreement that such change of position is due in part to active migration of the cells themselves and in part to unequal growth and change of form of the embryo. In some cases the germ-cells are seen during a part of their history to be distinctly amoeboid, an observation which suggests how this migration is accomplished. By far the greater number of writers, however, have spoken of them as of rounded form through at least the greater part of their history. Allen ('06) suggested that possibly even these rounded cells may be able to execute migration by slight movements insufficient to give them distinctly amoeboid form.

*b. Path of migration.* The general course of migration is the same in all species so far studied, though there are differences of detail characteristic of each species.

*c. 'Lost' and degenerate germ-cells.* An interesting observation is that in many cases studied a considerable proportion of the early germ-cells for some reason fail to complete the migration and find lodgment in some other part of the body. The fate of these aberrant cells has not been followed, though there is evidence that many of them degenerate. Beard ('02) found in the skate that some of them segment to form little clusters of cells which led him to believe that very probably the further development of an occasional one may produce teratomata or dermoid cysts.



### 3. *Distinctive Characters of the Early Germ-cells*

A consideration of the features which characterize the early germ-cells is of interest and importance. It is desirable to see what are the earliest differences which have enabled various workers to recognize them, to distinguish them from the body cells among which they lie, and to see what light we may thus get upon their nature, manner of setting apart, and relation to the soma.

It is common to speak of the early germ-cells as large, rounded cells which stain faintly. They are also generally found to retain yolk spherules long after they have been absorbed in the surrounding cells. We have already seen that the earliest gonocytes are found associated with entoblast, and as a general characteristic of these cells it is often stated that they resemble entoblast. Another common description is that they are cells that retain their primitive characters for a considerable time. All these are but different ways of describing the same condition. The absence of mitotic figures during a considerable period is also commonly remarked, and it is this feature that accounts for their larger size.

The presence of a considerable amount of yolk in their cytoplasm often obscures the nucleus, so that it is difficult to study its details. Several observers state, that in early stages, they can detect no nuclear differences between the germ-cells and body cells other than the differences in size which early becomes manifest. In some cases it has been noted that in later stages the nuclei of the former stain less deeply than those of the surrounding body cells. Beard ('02 c) found that the nucleus of the germ-cell is often bilobed. I do not recall that any other writers mention this feature, so I cannot tell how general it is.

One other feature of the germ-cells should not be forgotten; that is, their amoeboid shape during a part of their history. This feature, of course, is directly related to the ability of the cells to execute independent movement.

From a study of the literature it appears that the differences which mark the germ-cells of vertebrates, arise gradually, and are due more to changes in the surrounding cells than to changes

in the germ-cells themselves. In no case has there been found any conspicuous difference, arising suddenly as in the case of a few well known species of invertebrates.

## II. OBSERVATIONS ON *LOPHIUS PISCATORIUS* L.

### 1. *Material and Methods*

The fish which is the subject of this study belongs to the order *Pediculati* (the anglers), and is known locally by a number of names such as 'fishing frog,' 'goose fish,' etc. It is common in the shallow waters of the North Atlantic on both American and European sides, ranging as far south as Cape Hatteras and the Mediterranean. The eggs from a single female, estimated to number from forty to fifty thousand, float near the surface of the water, inclosed in a ribbon-shaped gelatinous mass a foot wide and 30 or 40 feet long.<sup>2</sup>

Alexander Agassiz describes the egg mass as an enormous ribbon from 2 to 3 feet broad and 25 to 30 feet long.<sup>3</sup> His figures and account of the newly hatched fish agree well with the material used in this study. He figures the eggs but gives no record of their size and I have had no opportunity to measure living ones; but from eggs in a good state of preservation they appear to measure about 1.7 mm. in diameter.

The greater part of the material used in this study was from the coast of Maine, collected and preserved by the Harpswell Laboratory of Tufts College. A series of fourteen vials of embryos of successive stages were studied. In the following account these stages are referred to by the consecutive numbers 1-14. In stage 1 cleavage has advanced to a point at which the germ-disc in several cells in thickness, but has not yet begun to grow around the egg. Owing to the spawning habits of *Lophius*, it is only by chance that the early cleavage stages are collected, the segmentation always being somewhat advanced when the eggs

<sup>2</sup> Jordan, David Starr, 1905. A Guide to the Study of Fishes.

<sup>3</sup> Agassiz, Alexander, 1882. On the Young Stages of Osseous Fishes, Part 3. *Proc. Amer. Acad. Arts and Scis.*, 17.

are found. However, as will be seen later, these earlier stages would probably not have added anything to the knowledge of the germ-cells in these studies.

The material was fixed in corrosive sublimate acetic; the fixation is good, as evidenced by the mitotic figures. The material, however, was preserved in formalin, which somewhat impaired the staining power of the tissues and prevented the use of certain stains it was desired to use. In most respects, however, the reaction to stains was satisfactory. A single vial of embryos of stage 14, preserved in alcohol, gave somewhat better results.

The stain which gave the best general results was iron-haematoxylin, with eosin or bordeaux red as a plasma stain. Best results were secured by using the 'long method' for iron-haematoxylin—twenty-four hours in 4 per cent iron-alum and an equal time in the 1 per cent aqueous haematoxylin solution. A set of embryos left by accident for thirty-five days in the haematoxylin solution was remarkably clear for showing cell boundaries, which were very indistinct in all other preparations. Delafield's haematoxylin gave fair results but was not so generally satisfactory as the iron-haematoxylin.

The main part of the study was from serial transverse sections 6 microns in thickness. A few embryos were sectioned in frontal and sagittal planes. It was found more satisfactory to imbed and section the whole egg than to dissect off the embryo, the yolk being so soft that it did not prevent the cutting of thin sections. To allow free access to fluids, an opening was always made in the egg membrane, but even with this care it was almost impossible to pass from absolute alcohol to the clearing fluid without considerable shrinkage and wrinkling of the egg. I do not think, however, that the tissue elements of the embryo were more affected by shrinkage than they would have been if the yolk and egg membrane had been removed.

## 2. *Distribution and Migration*

A clear idea of the part of the embryo where germ-cells are found can best be had from a study of cross sections. Figs. 1-11

are diagrams prepared from sections through corresponding parts of embryos of various ages. The general location of germ-cells is marked by solid black circles. These figures do not show the actual number or position of germ-cells in any one section, but rather present a composite picture from several sections.

The youngest embryo in which I was able to recognize germ-cells was of stage 6. At this time the blastoderm has not quite half covered the yolk. Fig. 12 is an outline drawing of such an embryo, and fig. 1 a cross-section. In this stage the ectoblast is already considerably thickened but the thickening has not become narrowed to form the well defined nerve cord of embryos a little older. Below the ectoblast, lying upon the periblast, is the primitive entoblast, made of rounded, loosely aggregated cells, which show a rather indistinct arrangement in two layers, the future mesoblast and entoblast (fig. 21).

In embryo '6 F,' two germ-cells were observed, one at either side, well toward the margin of the primitive entoblast, in position indicated by the solid black circle to the extreme right of fig. 1. Fig. 21 is a drawing of this cell and its surroundings. In embryo '6 D,' just a little more advanced than the preceding, there were observed two germ-cells, also in the entoblast, but near the median line of the embryo (figs. 1, 12 and 22). In no other embryos of this stage of development, was I able to recognize germ-cells. However, in both of these embryos, as well as in others of the same age, there were distinguishable quite a number of cells which may possibly be germ-cells that have not attained the characters which make them easily recognized from this time on. The position of these cells is indicated by the open circles in figs. 1 and 2.

Embryo '6 E' is somewhat more advanced than either of the above. The entoblast and mesoblast are fairly distinct and the notochord is separated. The mesoblast consists of about two layers of cells; the entoblast of a single layer. The cells of both of these germ layers are rounded and loosely aggregated. In this embryo, seven germ-cells were distinguished, distributed in both the entoblast and mesoblast (figs. 2 and 23).

In embryos a little older, the germ-cells are more clearly marked, owing chiefly to changes in the surrounding tissues. In embryo '7 H,' the mesoblast plates are considerably thickened and the cells have become more densely packed and so more angular, but there is as yet, no separation into myotome and lateral plate. In this embryo there were recognized fourteen germ-cells, all in the mesoblast and all in that part of it which will become myotome when the separation takes place (fig. 3, cf. figs. 2 and 4).

In embryo '7 F,' the myotome is very definitely separated from the lateral plate. There were counted fourteen germ-cells, of which twelve were in the myotome and two in the lateral plate (fig. 4). Embryo '7 Fr' was sectioned in a frontal plane and the myotome was seen to be also clearly separated from the lateral plate but not distinctly segmented. In this embryo nineteen germ-cells were recognized, all but one of which were in the myotome (fig. 13). In each of these embryos, nearly all of the germ-cells occupied the dorso lateral part of the myotome.

In embryos of stage 8, several myotome segments are clearly formed (fig. 14). By this time, the germ-cells have almost all migrated from the myotome into the lateral plate (figs. 5 and 14). A careful study of embryo '8 E' showed a total of thirty-nine germ-cells, all but one of which were in the lateral plate, near its inner margin. In embryo '8 D,' with thirty-six germ-cells, two yet remained in the myotome. From a study of embryos it appears that the migration from myotome to lateral plate must be a rapid one and accomplished by the active migration of the cells themselves.

Embryos of stage 9 show the gut pretty definitely formed, and the beginnings of the coelomic cavity within the lateral plate. When the separation of the lateral plate into somatic and splanchnic layers takes place, the germ-cells are left in the splanchnic layer, or close to the median margin of the lateral plate where no separation has taken place. In no embryo of this stage did I observe one in the somatic layer (fig. 6). In this and the following stages the number of germ-cells is about the same as in stage 8. The actual numbers will be given in a later part of the account.

About this time the germ-cells migrate among the cells of the splanchnic mesoblast, toward the medial border of the coelom, and thence upwards into the somatic layer. Different stages of this process are shown in figs. 7 and 8. Stage 11 marks the first indication of the formation of the pronephric duct, which in stage 12 has become separated from the coelomic mesoblast throughout the greater part of its length. About the same time begins the growth of the coelom around the gut (figs. 8 and 9). In embryos of stage 12 the germ-cells have reached the dorsal wall of the coelom and are for the most part close to the pronephric duct (fig. 9). In one embryo of this stage a single germ-cell was seen still in the splanchnic layer and near the side of the coelom (fig. 9). This is the only aberrant germ-cell observed with certainty in any embryo.

About stage 10 the blastopore closes (fig. 15), and at stage 12 the embryo is ready to break from the egg membrane (fig. 16). Embryos of stage 12 are about 2.7 mm. in length and curve half way around the egg. Stage 13 includes the newly hatched fish with the yolk sac still large (fig. 17). Immediately after hatching the growth of the embryo is rapid, owing probably to the absorption of water. The greater size is due largely to increase in the amount of mesenchyme (cf. figs. 9 and 10). There also begins at this time, a rapid increase in length. Fig. 17 shows an embryo of this age measuring about 4 mm.

It will be noticed that one of the very prominent changes at this period is the very great and sudden enlargement of the coelom. During this process the coelomic wall becomes very much thinner, and this leaves the germ-cells standing out prominently on the dorsal wall of the coelom, just below the pronephric duct. These great changes in the embryo at the time of hatching cause scarcely any change in the position and distribution of the germ-cells, other than a noticeable scattering (fig. 10).

By the time stage 14 is reached the yolk-sac is almost absorbed and the fish has attained a length of 6 mm. (figs. 11 and 18). The changes in internal structure have been great. The gut has become larger and longer, forming one coil, and the coelom has entirely surrounded it producing dorsal and ventral mesenteries.

The ventral mesentery, however, is of very short duration. At this stage, all of the germ-cells are located at the base of the mesentery where they are more closely aggregated than in stage 13 (fig. 11). A study of stages a little younger than this, shows that the germ-cells are, in large part, pulled into position at the base of the mesentery by the folding of the coelomic wall which forms the mesentery. It seems probable, however, that there is also at this time an active migration of the germ-cells themselves to bring about their closer grouping.

The germ-cells are now in the position of the permanent germ-glands (ovary or testis) and the studies on *Lophius* were not carried beyond this point. The studies of Eigenmann ('96) and Böhi ('04), on other Teleosts, show clearly enough the fate of these cells. These writers have traced them in the gonads up to the time of sex differentiation and have found that the functional sex-cells arise as their lineal descendants.

In the foregoing account we have traced the germ-cells from their position in the primary entoblast, where they were first distinguished, till they have reached the place of formation of the gonads. It should be borne in mind, however, that this study does not assume to show the actual origin of the germ-cells in *Lophius*. This account simply takes up the history from the time at which I was first able to recognize them. Reasons will be given later, for believing that they are set apart at a time earlier still in embryonic history.

There remains yet to be described, the antero-posterior distribution of the germ-cells. Some idea of this may be had by reference to figs. 12-18, where the actual number of germ-cells and their position are indicated by black dots upon outlined drawings of the embryos. Each drawing pictures their actual position in a single embryo. To determine their distribution, a camera lucida drawing was made of each section in which germ-cells were found. The position of each cell was then plotted according to the section in which it was found. From a study of twelve embryos it is quite evident that the arrangement of the germ-cells is not alike in any two embryos of the same age so that groupings very different from those figured are found, though as

nearly as could be determined, the cases figured represent average conditions. Usually most of the germ-cells are grouped in a more or less compact mass, with a few scattered ones in front of this group and others distributed for some distance from the main mass in all embryos studied (figs. 17 and 18). It would be of interest to know whether these scattered cells take part in the formation of the gonad or whether they degenerate.

Between stages 8 and 13 there is a gradual increase in the antero-posterior extent of the germ-cells. This is correlated with the increase of length of the embryo, or rather with the growth of the coelomic mesoblast in which they lie. The elongation of the segmental structures does not have any influence. In all embryos, the anterior extent is about co-incident with the forward limit of segmentation, and the posterior not far forward of the termination of the gut.

It should be noted in this connection, that the germ-cell groupings on the two sides of the embryo is not symmetrical. The groups of one side do not necessarily correspond to those on the other.

### 3. *Number of Germ-cells*

In order to find if there is in *Lophius* the period of rest from division of the germ-cells common in vertebrates, a careful tabulation of the germ-cells in embryos of each age was made. Though their number in *Lophius* is small, to make an accurate count in any embryo is not easy. In the early stages especially the danger of overlooking some of them is very great. There is also, when the nucleus is cut and appears in two adjacent sections, the danger of counting the same cell twice. To avoid this danger, camera lucida drawings were made of each section in which there were germ-cells. These drawings were made on transparent paper so that drawings of adjacent sections could be superimposed, and thus corresponding halves of nuclei properly related, and the danger of error in counts made by this method is, I believe, small. After making a few counts by this method it appeared that counts made previously by less careful methods were of no value.



The following table presents the results of these counts for a number of individuals (these denoted by letters) of each stage:

TABLE 1.  
*Showing number of germ-cells*

STAGE OF EMBRYO	6	7	8	9	10	11	12	13	14
Number of germ-cells	F.2	H.14	D.36	J.36	D.31	D.40	G.34	C.30	E.39
	D.2	F.14	E.39		E.29		C.30	E.28	B.38
	E.7	Fr.19						W.45	D.38 C.35
Average number	3 +	15 +	37 +	36	30	40	32	34 +	37 +
Average of stages	8-14		35.2						

A study of the above table shows, first of all, that while the number of germ-cells in all embryos is not the same, yet in stages 8-14 there is no general increase, the average number in stage 8 being the same as in stage 14. The number of embryos studied is too small to allow us to determine with accuracy the general average or the range of variation, but it is sufficient to show that there is no increase in number during this period. That there is no increase in number might be inferred from the absence of mitoses in these cells during the same period. In stage 14, however, there are seen changes in the nuclei of the germ-cells which indicate that they are in preparation for division, so that it is very probable that in fish a little older the number would be greater. Stage 14 may then be considered as, in all probability, marking the close of the rest period of the germ-cells.

The variation in the number of germ-cells in different embryos is also very evident. In the fifteen embryos of stages 8-14 in which careful counts were made, the extremes are 28 and 45, by far too great a variation to be explained as inaccuracy of count. This is not in accord with "the numerical law of germ-cells" as expressed by Beard ('02 d), which demands that the number during this period shall be the same in all individuals.

From a study of the numbers given in the above table, one would conclude that stages 6 and 7 mark the early multiplication period, and that there is, in these stages, an actual increase in the number of germ-cells. A study of the actual specimens, however, brings us to the conclusion that there is no increase in number during these stages. For reasons to be given later, it is believed that the full number of the rest period is attained somewhat earlier, probably in stages 5 and 6, though we are unable at this time to distinguish them from other cells in the lower layer of the embryonic area, and that during stages 6, 7 and 8 they undergo changes which make them recognizable. This, then, is not a multiplication period, but an early part of the period of rest during which the germ-cells first attain the characters by which they may be recognized.

#### 4. *Distinctive Characters of the Early Germ-cells*

The germ-cells of most vertebrates so far studied are, up to quite a late stage, filled with deeply staining yolk spherules which cause them to stand out prominently among the surrounding cells. In my preparations they are marked in no such conspicuous manner. Whether this is due to methods of fixation I do not know, but the absence of yolk has made both necessary and possible a somewhat careful study of other features of the cell.

An account of the structure of the germ-cells of *Lophius* had best be given in three parts, corresponding to three well-marked periods in the history of the cells themselves. These I will call 'early', 'middle', and 'later' periods which mark three divisions of the period of rest.

*a. Middle period.* For the sake of clearness the middle period will be described first. This includes stages 9-13, during which time the germ-cells do not undergo any appreciable change (figs. 29-33). During the same time, however, the surrounding cells, those of the coelomic mesoblast, undergo very marked changes. In stage 9, the germ-cells are but little greater in volume than the surrounding cells (fig. 29) but during further development they remain of the same size, while the coelomic cells become smaller

and flattened and their nuclei decrease in size, so that by stage 13 the germ-cells stand out prominently in the peritoneum (fig. 33).

During this period the cells of the coelomic mesoblast are angular in outline, while the germ-cells are rounded. Cell boundaries between somatic cells are very indistinct and at times cannot be distinguished at all, but the boundaries of germ-cells are always fairly distinct. The cytoplasm of the germ-cells does not stain deeply, so that they are always paler than the surrounding cells, but not conspicuously so. These pale cells must not be confused with other lightly staining cells scattered about in the embryo, nor with the glistening white cells common in the entoblast. It was also noticed that when a deep eosin stain was used the germ-cells frequently took on a yellowish hue, but this was not conspicuous.

Turning now to the nucleus we find some points of interest. Figs. 29-33 show that the nucleus of germ-cells is noticeably larger than that of other cells. This is already quite well marked in stage 9 (fig. 29), and the difference becomes more pronounced in each succeeding stage, because the nucleus of the somatic cells is becoming continually smaller while that of the germ-cells remains of constant size. The shape of the nucleus also is different in the two kinds of cells. In somatic cells it is usually quite regular, its outline being circular or elliptical, while in germ-cells it is decidedly irregular, being commonly quite deeply indented at one side or having a still more irregular shape. This character of the cell is shown in figs. 29-33, but in some cases the nucleus lies in such a position that its irregularity of contour cannot be shown in drawings.

The chromatin is the one conspicuous feature in which the two kinds of cells cannot be seen to differ. In both the greater part of the chromatin-linin reticulum is next the nuclear membrane, and in both it also covers the plasmosomes (nucleoli).

Perhaps the most striking difference between the two kinds of cells is seen in the plasmosomes, and this is the feature which more than any other was used in distinguishing them. In the germ-cells there are two small plasmosomes, usually quite widely

separated, while in other cells of the embryos the plasmosome material forms two large masses or a single mass of still greater size. The amount of plasmosome material is very much greater in body cells than in the germ-cells. The plasmosomes of the somatic cells are commonly near the center of the nucleus while those of the germ-cells are nearly always on or near the nuclear membrane (figs. 29–33).<sup>4</sup>

*b. Later period.* Under this heading are described changes seen to be taking place in stage 14 and to a less extent in stage 13. In stage 14 the germ-cells occupy a position on either side of the base of the mesentery. They are much more closely aggregated than in earlier stages, and surrounding them are flattened cells derived from the peritoneum, undoubtedly the beginning of follicle cells. We have here very clearly the beginning of the formation of the permanent sex-gland.

The changes of the germ-cells themselves at this period are well marked. A group of these cells is shown in fig. 34, three of which have changed considerably while the remaining two are still in the condition common in the earlier period. The nucleus is no longer irregular and shrunken in appearance, but has become round and full and is much larger than that of the earlier cells. The actual size of the whole cell has changed little, if at all, so that the nucleus occupies a much greater proportion of the cell space than before. Another conspicuous change is the thickening of the chromatin-linin reticulum; this causes the nucleus to stain more deeply. The two plasmosomes of former stages have now become fused into one single mass and its total volume has increased very considerably. These changes are probably to be followed shortly by cell division, so that in embryos a little older we should expect to see many of these cells in mitosis. These cells are evidently the primary oögonia (or spermatogonia) as direct descendants of which the functional sex-cells of the mature fish arise.

<sup>4</sup> A prominent feature of the drawings of these stages, also prominent in the sections themselves, is the large amount of dark brown or black pigment. Pigment cells may be distinguished as early as stage 7. From this time on pigment steadily increases in amount, till at the time of hatching it is a very prominent feature of the young fish. (figs. 17 and 18).

*c. Early period.* Under this heading will be described the germ-cells of embryos younger than stage 9. About this period centers the greatest interest of the present studies, because such stages mark the only gap in our knowledge of the germinal cycle of vertebrates. In these early embryos it becomes increasingly difficult to distinguish between germ-cells and somatic cells, and in no vertebrate has the germ-cell line been traced back to the unsegmented egg. In *Lophius* germ-cells were not recognized in embryos younger than stage 6.

The germ-cells of this period bear a very close resemblance in all features to those of stages 9–13 previously described. Their outlines are rounded and more distinct than those of body cells; their nuclei are irregular in shape and each has two small plasmosomes quite far apart and close to the nuclear membrane as in older embryos. The germ-cells are, however, in the early period but little larger than other cells and stain almost as deeply, which conditions make them difficult to recognize. In searching for them in the sections the difference in the plasmosomes is the only feature which readily strikes the eye and accordingly it was to this feature more than to any other that attention had to be directed in tracing the germ-cells in the early stages.

In stages 1 and 2 the plasmosomes of all cells are small (figs. 19 and 20) suggesting the germ-cells of embryos a little older, and I first thought that possibly the condition in the germ-cells was primitive, persisting from these early stages. This seemed the more reasonable because it is a matter of general observation that the germ-cells of vertebrates actually do retain primitive characters longer than other cells of the embryo. Careful search, however, failed to reveal in any embryo of stages 3, 4 or 5, a single cell which showed this or any other recognizable character of germ-cells. Their line could not be traced back by this character as I had hoped it might.

In all embryos of stages 6 and 7 a small number of germ-cells could be recognized, and once found there was seldom any difficulty in being certain about their identity, but the most careful search failed to reveal in embryos of these stages as many of them as there were in older ones. A glance at table 1, page 578, shows

that there is a consistent increase in the number of germ-cells during stages 6, 7 and 8, the average number in embryos of each of these ages being respectively 3, 15 and 37. As previously remarked, this increase in number might reasonably be interpreted as an indication of the early multiplication period, but reasons will now be given for thinking that this is not the case but that this is in reality the early part of the period of rest.

We may first note that during this period none of these cells were actually observed in division and this indicates that this is not a period of multiplication. This observation, however, is not conclusive because it is entirely possible that such cells could not be recognized during mitosis because of the disappearance of their distinctive features.

The relative size of the germ-cells and body cells furnishes more satisfactory evidence on this point and indicates quite clearly that the period of rest begins at about stage 6. It has already been seen that in later stages the germ-cells are decidedly and increasingly larger than the cells surrounding them and that this difference of size is due to the arrest of cleavage of the former. The beginning of the difference of size marks the beginning of the period of rest. In stage 6 the few germ-cells recognized do not differ visibly in size from other cells of the primary entoblast among which they lie (figs. 21, 22 and 23). In stage 7, however, their nuclei are slightly but noticeably larger than those of surrounding cells (fig. 28), while in stage 8 the difference is more pronounced (fig. 29). The arrest of cleavage has already resulted in a difference in size which indicates clearly that the apparent increase in the number of the germ-cells in these stages is not due to an actual multiplication.

During the study of these stages the explanation of the apparent numerical increase came in an entirely unexpected manner. Phenomena were observed which led to the conclusion that the small size of the plasmosomes of the germ-cells is not primitive, but that during the early part of the rest period there is an actual reduction of their size by extrusion of substance from the nucleus. It is also in connection with this process that the nucleus attains its irregular shape, and not until this has taken place can the germ-

cells be distinguished. This explains the apparent increase in number during this period.

The nature of the evidence leading to this conclusion may be briefly stated as follows: In some of the germ-cells and in no other cells, of embryos of stages 6, 7 and 8 there was seen in the cytoplasm a small round deeply staining body, about the same size as one of the plasmosomes within the nucleus, or a little smaller (figs. 21-23, 25-28). This I believe, is a mass of plasmosome material which has been separated and cast out of the nucleus.

Only a few cells showing this feature have been figured but the number might have been multiplied because in every embryo of these stages a considerable proportion of the germ-cells had this body in the cytoplasm. On account of the manner of preservation of my material it was not possible to apply any stain which would show positively whether this body was of the same nature as a plasmosome within the nucleus, though the two stained similarly with the stains employed.

The following table gives for embryos of different ages the number of germ-cells in one column, and opposite each, the number in which the extruded plasmosome was observed.

TABLE 2.

*Showing distribution of observed cases of extruded plasmosome*

STAGE OF EMBRYO	6		7		8		9	
	Emb.	g.c.-ex.	Emb.	g.c.-ex.	Emb.	g.c.-ex.	Emb.	g.c.-ex.
Number of germ-cells observed and numbers showing extruded plasmosome	D	2-2	H	14-10	D	36-5	J	36-1
	F	2-2	F	14-8	E	39-3		
	E	7-4	Fr	19-12				

In none of the above cases was the actual process of extrusion observed, which indicates that the process must occupy but a short period of time. There were cases observed, however, which leave little doubt as to the nature of the extranuclear body and the manner of its extrusion. This body was observed in no cell in which both plasmosomes were of large size, but was quite

frequent in cells with two small ones (figs. 22, 23, 26-28). It is also important to note that it is not infrequently seen in the cytoplasm of cells with one large plasmosome and one small one (figs. 21 and 25). Such observations as these give good reason to believe that the body is actually an extruded plasmosome, because at the time of its appearance the nuclear plasmosomes become smaller. Fig. 26 shows a cell in which the process of extrusion is apparently just completed. The extruded body lies close to the nuclear membrane, and directly opposite it, just within the nucleus, lies one of the small plasmosomes, as if the extranuclear part had but recently been separated from it. Fig. 25 shows a case somewhat similar, with this difference, that but one of the nuclear plasmosomes is of small size, and the extra-nuclear one lies opposite this. It is also important to notice that there was no clear case observed of a cell which had two of these bodies in the cytoplasm.

From such conditions as the above it appears that during extrusion of material, each nuclear plasmosome moves to the nuclear membrane and there gives out part of its substance into the cytoplasm. The fact that in later stages the plasmosomes of germ-cells are in contact with the nuclear membrane gives support to this view. It is also evident that the reduction of both plasmosomes is not simultaneous, but that each gives out part of its substance at periods sufficiently far apart to allow of the disappearance of the first one before the extrusion of the second. The other possible mode of extrusion is that within the nucleus there occurs a division of each plasmosome followed by the extrusion of one fragment of each from the nucleus. The only instance I have observed which may possibly indicate such a division within the nucleus is drawn in fig. 24. In this nucleus there is a third body which may possibly be a fragment of a plasmosome about to be cast out of the nucleus. This single case, however, is not clear enough to be of great significance.

A study of the distribution of the cases as shown in table 2, page 584, indicates that the process of extrusion is most active at stage 7, because at this stage there are more cells with an extruded plasmosome than at earlier or later stages. It is



evident that the process does not take place in all cells at the same time, but that it begins at stage 6 with a few cells and continues until its completion at stage 8. The important point to be made clear in this connection is that it is only as this process progresses that the germ-cells can be recognized and not until its completion can the full number be distinguished.

A study of the germ-cells of this period indicates that though the full number cannot be recognized until stage 8 the actual beginning of the rest period is somewhat earlier, possibly at stage 6 or at the youngest stage 5. That the period of rest does not extend back into younger embryos is evident from the larger size of the cells of these stages. It is in embryos of these stages, 5, 4, etc., that we must look for the early multiplication period. At present we are unable positively to identify any germ-cell previous to the extrusion of plasmosome material from the nucleus, yet they are unquestionably set apart as germ-cells before this process takes place.

In stage 6 these cells are to be sought in the primary entoblast where the first cases of extrusion are observed. In this part of embryos of stage 6 there are some cells, not very clearly distinguished from the others, which may possibly be germ-cells prior to the extrusion of plasmosome material. I refer to some cells with nuclei less rounded than the others and having two plasmosomes somewhat separated. Two of these are shown in fig. 21, *g.c.*? The nuclei of these cells are somewhat indented but are by no means as irregular as those from which plasmosome material has been extruded. Compare these two cells with the germ-cell in the same drawing in which one plasmosome has undergone decrease of size, and the one in fig. 22 in which both have undergone the process. The location of these possible germ-cells is indicated by open circles in figs. 1 and 2. I do not point to these positively as germ-cells, but simply call attention to the possibility that these are the cells which a little later take on the more obvious distinctive characters of germ-cells.

From the facts presented in the preceding pages we may draw the following conclusions:

1. There is an extrusion of plasmosome material from the nucleus of the primary germ-cells. This marks the beginning of the more obvious characters which enable us to distinguish the germ-cells.

2. This extrusion of plasmosome material takes place very shortly after the beginning of the period of rest.

3. The process does not take place in all germ-cells simultaneously.

4. The reduction of both plasmosomes does not take place at the same time.

### 5. *Discussion and Conclusions*

*a. Means of migration* It is now in order to inquire by what means the change of position of the germ-cells of *Lophius* is accomplished. Two possible means are suggested by all investigators—active migration, and passive change of position due to growth of surrounding tissues. In *Lophius*, both of these forces seem to be at work. The pulling of the germ-cells into the base of the mesentery at the time of its formation is, for example, brought about by growth of the tissue, while their closer grouping at this time is very probably due to an active migration. The change from the myotome to the lateral plate is an active migration, and quite a rapid one, and the change of position from splanchnic to somatic mesoblast at a period later, can only be accounted for in the same way. It also seems probable that at an earlier period (stage 6), some of the germ-cells at least migrate from a lateral to a more median position in the primary entoblast.

It is very commonly recognized that the migrations of germ-cells, in both vertebrates and invertebrates, are accomplished by amoeboid movement, and cells very distinctly amoeboid in shape are often recognized. In *Lophius*, during most of the period of migration, the germ-cells are decidedly rounded in outline. The only ones which might be considered amoeboid, were observed in embryos of stages 7 and 8, which, it will be remembered, include the period of active migration from the myotome into the lateral plate. In other stages it is not unreasonable to

suppose, as suggested by Allen ('06), that slow movement of germ-cells among cells of surrounding tissues may be accomplished by slight movements, not sufficient to give distinct amoeboid shape.

*b. Path of migration.* The path followed by germ-cells in their migration to the genital ridge has been described somewhat differently for each species studied. These differences, however, are but variations of the same general course. In *Lophius*, but one feature of the migration seems worthy of comment—at one stage all of the germ-cells are found in the myotome. When I first saw them in this part of the embryo, I thought possibly there was a mistake about their identity, because, at this stage it is no easy matter to recognize them. Study of several embryos of stage 6, 7 and 8, however, showed that these were really germ-cells, and that in some embryos none could be found in any part of the body except in the myotome. It showed also that the natural path of migration was through the myotome (cf. figs. 2-5).

The writer does not attach any special importance to this feature of the migration path in *Lophius*. It does not seem to make any difference what parts of the embryo are traversed in the migration nor in what parts of the body they may find temporary lodgment. The path varies in different animals, but in each species it is fixed and definite.

The question why they follow a definite path in one species and a somewhat different one in another cannot at present be answered in full. The details of the path seem in some way to be correlated with the course of development of the embryo, but the exact relations do not appear very clear. It might be expected that the path of migration would be the shortest and most direct course to the place to be reached. The course does not, however, appear to be determined in this way, and in *Lophius* shows several deviations which cannot be accounted for by such a simple explanation. We see the first possibility of complications when we note that the migration is in part due to the cells themselves and in part to the tissues of the embryo.

*c. Segmental relations.* It is of interest to inquire whether at any time, in *Lophius*, the arrangement of the germ-cells is seg-

mental, corresponding to the segmentation of the paraxial structures of the embryo. This question is the more pertinent, because of the view, held by Van Wijhe ('89) and some others, that the germ-cells in all vertebrates arise from the gonotome, a part of the segmented mesoblast. The observations on *Lophius* do not give any support to this view, though the presence of germ-cells in the myotome at an early stage might be so interpreted. The myotome, however, is not their place of origin in *Lophius*; they are observed before the myotome is formed, in fact before the mesoblast itself is definitely separated, and, as we have seen, there is reason to believe that their actual origin is still earlier. They cannot be said to be mesoblastic in origin and certainly they are not segmental.

In no later stage, moreover, does their grouping appear to bear any relation to the segmentation of the embryo. At first I thought possibly there might be such a relation, but study of several embryos of different ages showed that the groups of germ-cells in no way correspond to the metamerism of the embryonic trunk.

*d. Criteria of germ-cells.* During this study, the question has frequently suggested itself, how do I know that through all stages I am dealing with the same group of cells? What is the evidence that the cells here described do not represent merely a cyclical stage of ordinary cells? There are three kinds of evidence which support the view that through the different stages, we have to do with the same group of cells. These are as follows: First, constancy of size and appearance. The stability of size and structure of these cells, through a period marked by great change in all other cells of the embryo has already been sufficiently emphasized. Second, constancy of number. In stages from 8 and upwards, the number, though varying somewhat in different embryos, is constant enough to give strong support to the view that we are dealing with a definite group of cells. During this period, the size, structure and number of these cells is by far the most constant and unchanging feature of the embryo. Third, correspondence of position. In embryos of the same age, these cells are always found, with minor variations, in the same part of the

embryo, and throughout the series of stages, they may be traced as following a perfectly definite path.

The next point is to determine whether these are really germ-cells. Are they actually the cells which later give rise to oögonia and spermatogonia? Though I have not traced them through the later stages, they correspond so well with cells which in teleosts, as well as in other groups of vertebrates, have been definitely traced to these stages, that there can be little doubt of their identity. It would not have seemed necessary to raise this question at all, were it not for the contention of some recent writers, that these so-called 'sex-cells,' of early stages, including the 'Ureier' of Waldeyer, are not germ-cells at all, but simply hypertrophied cells which later entirely degenerate.

*e. Period of rest of the germ-cells.* A period of rest from division of considerable duration seems to be general during early stages of the germ-cells of vertebrates. In some invertebrates, at least, the same is the case. A phenomenon of such general occurrence should be of some significance.

In all vertebrates examined this period corresponds to the time during which the germ-cells are in active migration, and it has been suggested that possibly the energy of the cells is expended in locomotion rather than in growth and cell division.

It has also been pointed out, that this period corresponds to the period of embryo formation. This relation is certainly an evident one in many forms, and may be of some significance. In *Lophius* it is very apparent. At stage 6, which probably marks the beginning of the period of rest, the formation of the embryo has scarcely begun. By stage 14, which probably about ends the period of rest, the organs and tissues of the embryo are quite definitely formed. Thus it appears, as suggested by others, that the germ-cells are early set apart, after which they rest while the embryo is being built for their reception. Such observations give support to the view that germ-cells are really primitive, generalized cells, early set apart so that they may retain the possibilities of development which become lost during histological differentiation of somatic cells. This may be the true reason for

the phenomenon of arrest of cleavage, but it in no way explains the mechanism by which it is brought about.

The conditions observed in *Lophius* point very clearly to this as a period of arrested activity of the germ-cells, during which, for some reason the metabolic processes of the cells are relatively low. The arrest of mitosis is of itself an evidence of suspended activity. The small size of the nucleoli is also in all probability an indication of the same condition. It is I believe quite generally agreed that the size of the plasmosome is in some measure an index of the activity of the cell. Whether the extrusion of a part of the plasmosome material from the nucleus, is also an evidence of lowered metabolic activity of the cell, I cannot say. It indicates at any rate some important physiological difference.

Every nucleus, at mitosis, gets rid of its plasmosome material. There are also cases on record of an extrusion of plasmosome material from the resting nucleus, and this extrusion seems dependent upon the activity of the cells, as if the substance accumulated so rapidly that there was need to get rid of it between divisions.

The conditions of extrusion from the resting nucleus of the germ-cell of *Lophius*, however, seems to be entirely different, and not at all a manifestation of high activity. In the germ-cells of *Lophius* there is for a long time after extrusion of plasmosome material no increase in the size of the nuclear plasmosome remaining. In the somatic cells during stages 6-14 the amount of nucleolar material produced in the resting nucleus and lost during the many divisions must be considerable, while during the same period little or none is produced by the germ-cells.

The shape of the nucleus is also a probable indication of suspended activity in the germ-cells. In very active cells it is likely to be very irregular, sending out amoeboid processes, which present a large surface for the exchange of substances between nucleus and cytoplasm. The irregularity of the nucleus of the germ-cells of these embryos is of a different nature. They present rather a shrunken appearance, as if cessation of activity had allowed the nuclear sap to diminish, leaving the nuclear membrane too large. This shrunken appearance, as pointed out above, first becomes

noticeable at the time of the extrusion of the plasmosome. It hardly seems possible that the volume of plasmosome material lost to the nucleus at this time is sufficient to account for the shrinkage, but possibly at the same time other substances are also lost, and the nuclear membrane is in such a condition that they are not renewed. More recent physiological research has shown that changes in permeability of the membranes of the cell have much to do in determining its physiological condition. It may be that during the rest stage, the germ-cells of *Lophius* are characterized by a condition of impermeability of the nuclear membrane.

The above discussion of conditions observed in these cells during the rest period offers still no explanation why this period of suspended activity begins, nor why, after a time it comes to an end. At its beginning, before there are any differences we can detect with the eye, there must be an unseen physiological difference which determines the future behavior of the cell. Whatever the nature of this difference it is one of the earliest of which we have evidence in the cleaving egg of *Lophius*.

*f. Nuclear processes in germ-cell segregation.* A process similar to the extrusion of plasmosome material from the nucleus of the germ-cells of *Lophius* has not been observed in any other vertebrate. As described in other vertebrates, the first differences between the two groups of cells arise gradually and seem due to changes in the body cells rather than in the germ-cells. In *Lophius*, on the other hand, the first differences which enable us to recognize the germ-cells arise suddenly by nuclear changes within them. This feature of the early development of *Lophius* recalls nuclear changes of a somewhat similar nature which have been observed at the time of the first segregation of the germ-cells of invertebrates of various groups. The best known of these cases is that of *Ascaris megalocephala* described by Boveri ('92) and since verified by many others. During each of the four first cleavages there is a throwing off of chromosome ends from the nucleus in a single blastomere. At the fourth cleavage the blastomere containing the undiminished amount of chromatin is the first germ-cell. Three other species of Nematodes



are now known to have similar processes. Kahle ('08) describes a process of a somewhat similar nature in the development of gall-gnats (*Cecidomyidae*). At the 8-cell stage the cell with the undiminished amount of chromatin is the first germ-cell. Giardina ('01) and Debaisieux ('09) described in the beetle, *Dytiscus*, a loss of nuclear material during oögenesis. A single indifferent cell of the egg tube undergoes four divisions, during the first of which but half of the nucleus enters into the formation of the mitotic figure. The remaining half forms a separate mass which passes down entire to a single cell which at the fourth cleavage becomes a germ-cell, while the other fifteen daughter cells lacking this body are the follicle cells.

In the preceding cases it is the chromatin of the nucleus that is involved, while in others now to be described, the early nuclear changes have to do especially with the plasmosomes. Häcker ('97) found that in *Cyclops* during each of the first nine cleavages there is an emigration of plasmosome material from the nucleus of a single blastomere into the cytoplasm, where it disappears during the telophase. At the ninth cleavage this blastomere is the first germ-cell. The same writer ('92) described in the medusa, *Aequorea*, the extrusion of a plasmosome from the nucleus at about the time of the first maturation; this persists undivided in the cytoplasm up to the 64-cell stage; he did not trace the fate of this cell, but it seems probable in the light of other cases that this body may mark the first germ-cell. Silvestri ('06) found that in the parasitic wasp, *Lithomastix truncatellus*, the plasmosome is transmitted entire through the first two divisions to a single blastomere of the 4-cell stage. It is also probable that in this case this cell represents the beginning of the germ-cell line.

Elpatiewsky ('09) found that in *Sagitta* the first germ-cell is marked at the fifth cleavage by the presence in the cytoplasm of a 'besonder Körper' which has come down entire from the beginning of cleavage. He did not trace its origin but considered that probably it is nuclear. Buchner ('10) however, who later studied this point, considered that the body is the persisting chromatin of an ingested follicle cell. Hegner ('09) described the differentiation of the germ-cells of some Chrysomelid beetles as beginning



with the ingestion by particular cells of the granules of the pole-disc. Rather less complete descriptions of a similar process in some other insects had been given by earlier writers and mentioned by Hegner. Wieman ('09) studied the origin of the pole-disc in Chrysomelid eggs and found that the granules are secreted by the nurse cells and that the pole-disc is a portion of them which did not become transformed into yolk.

Among the preceding cases of very noticeable nuclear changes at the time of the segregation of the germ-cells there is no apparent uniformity between the different species but the relation of these different processes to the first segregation of the germ-cells is clear. In the light of these cases it is not surprising that in *Lophius* we should find nuclear changes of still a different sort. The extrusion of plasmosome material from the resting nucleus of the germ-cells of *Lophius* is an example among vertebrates of a process resembling the changes known to take place at the time of their segregation in a number of invertebrates.

### *Summary*

The separation of somatic- and germ-cells is one of the early differentiations in the segmenting egg of *Lophius*.

It has not been possible to recognize the germ-cells during the earliest stages of this process, but there is no question that the process is completed before the beginning of embryo formation.

The first clearly recognized germ-cells lie in the primary entoblast at a stage when the blastoderm has not quite half covered the yolk and the formation of the embryo has but begun.

The germ-cells pass into the mesoblast during its separation, or shortly thereafter.

The 'period of rest' of the germ-cells begins before the separation of the mesoblast and continues until after hatching. It corresponds to the period of 'embryo formation.'

The observations on *Lophius* do not cover the earlier period of multiplication of the germ-cells. The apparent increase during certain early stages, is due to changes in the germ-cells which make them recognizable.

During the early part of the period of rest there is an extrusion of plasmosome material from the nucleus of the germ-cells, but not from those of the body cells.

The period of rest is one of decreased activity of the metabolic processes of the germ-cells. They are in some way 'held back' from undergoing changes during embryo formation.

The germ-cells reach the position of the gonads by a migration which is in part active and in part passive.

In *Lophius* the path of migration passes through the myotome. This is no indication of a segmental origin of the germ-cells. There is no segmental arrangement of them at any time.

Shortly after the germ-cells reach the position of the gonad they show marked evidences of renewed activity.

Actual count shows that there is no increase in the number of the germ-cells during the period of rest.

The number of the germ-cells is not the same for all embryos of the same age but shows a very considerable variation.

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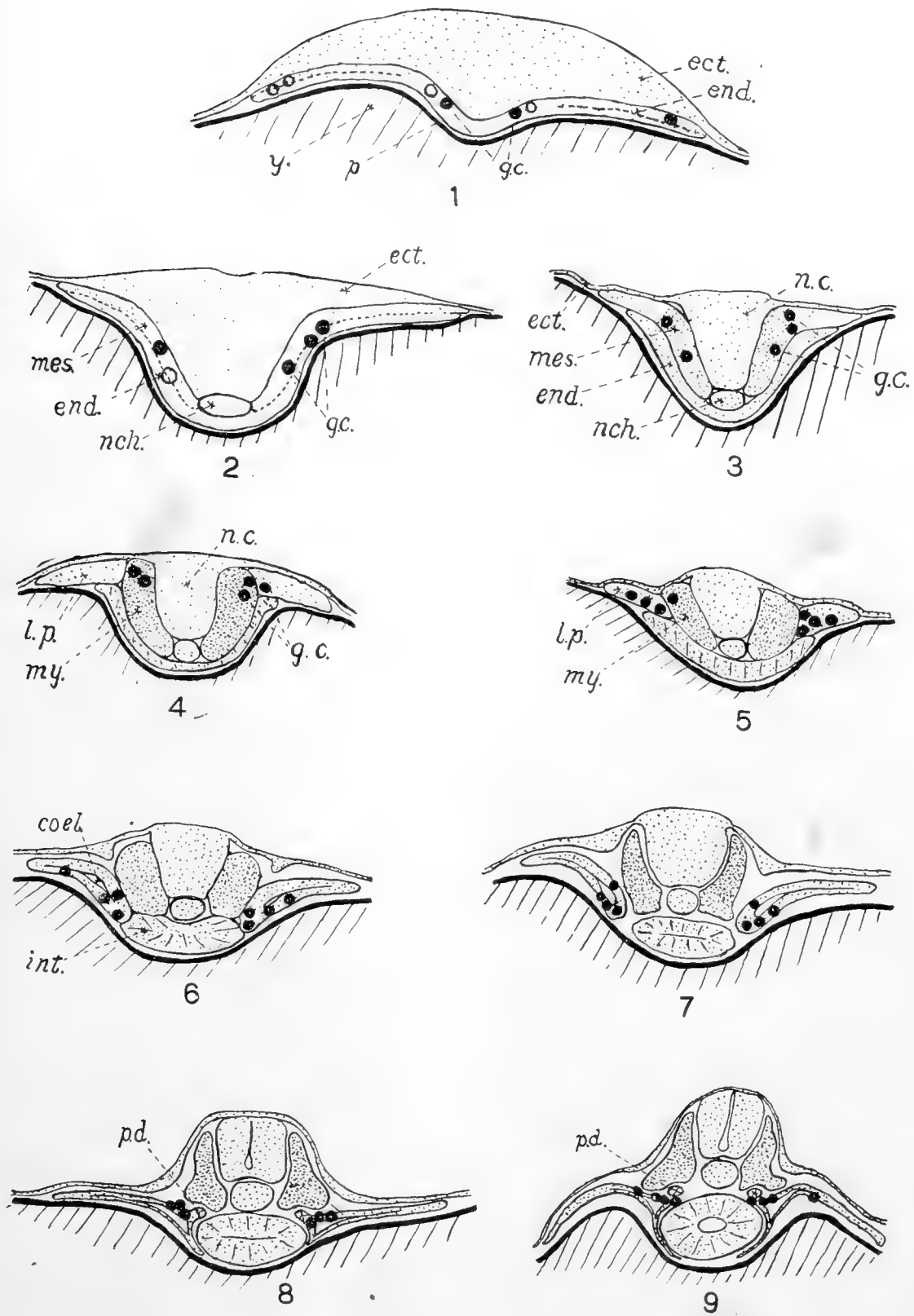
## EXPLANATION OF FIGURES

## ABBREVIATIONS

a.	anus	l. r.	limb rudiment
bp.	blastopore	m.	mesentery
coel.	coelom	m. a.	mesenteric artery
d. f.	dorsal fin	mes.	mesoblast
d. f. r.	dorsal fin rudiment	my.	myotome
e.	ear vesicle	n. c.	nerve cord
ect.	ectoblast	nch.	notochord
end.	entoblast	p.	periblast
ex.	extruded plasmosome	p. d.	pronephric duct
fol.	follicle cell	pel. f.	pelvic fin
g. c.	germ-cell	per.	peritoneum
g. r.	germ ring	pi.	pigment
h.	heart	pi. c.	pigment cell
he.	anterior end of embryo	p. p.	posterior end of embryo
int.	intestine	y.	yolk
l.	liver	?	supposed plasmosome fragment about to be extruded.
l. p.	lateral plate		

1-11 are diagrammatic representations of cross-sections of embryos of successive stages through the region of the germ-cells. The position of germ-cells is shown by solid black circles. All these drawings were outlined with the aid of a camera lucida and reduced to a magnification of 110 diameters.

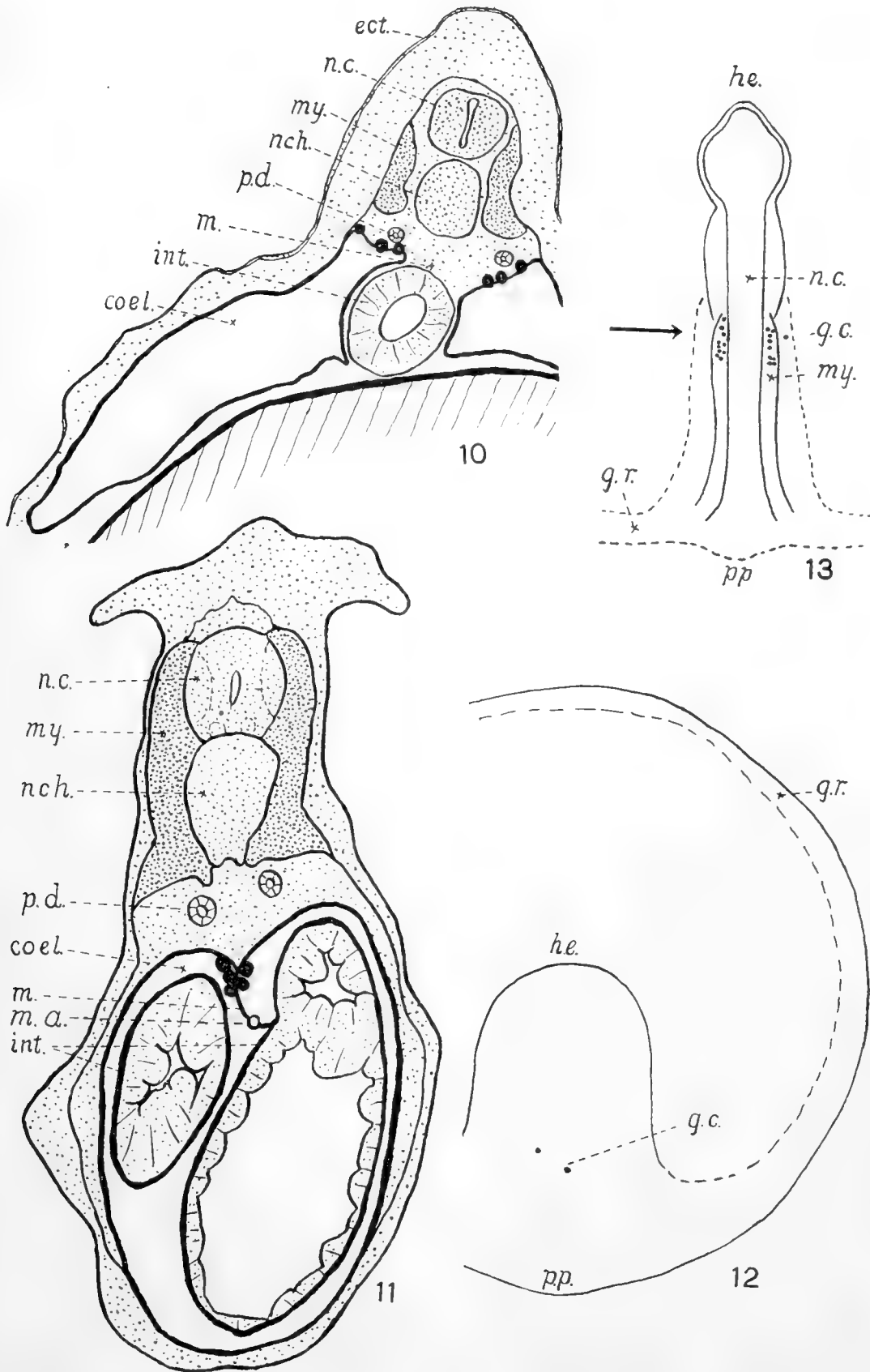
1. Cross section of embryo of stage 6.  $\times 110$ .
2. Cross section of an embryo of stage 6 a little more advanced than the one shown in fig. 1.  $\times 110$ .
3. Cross section of embryo of stage 7.  $\times 110$ .
4. Cross section more advanced embryo of stage 7.  $\times 110$ .
5. Cross section of embryo of stage 8.  $\times 110$ .
6. Cross section of embryo of stage 9.  $\times 110$ .
7. Cross section of embryo of stage 10.  $\times 110$ .
8. Cross section of embryo of stage 11.  $\times 110$ .
9. Cross section of embryo of stage 12.  $\times 110$ .





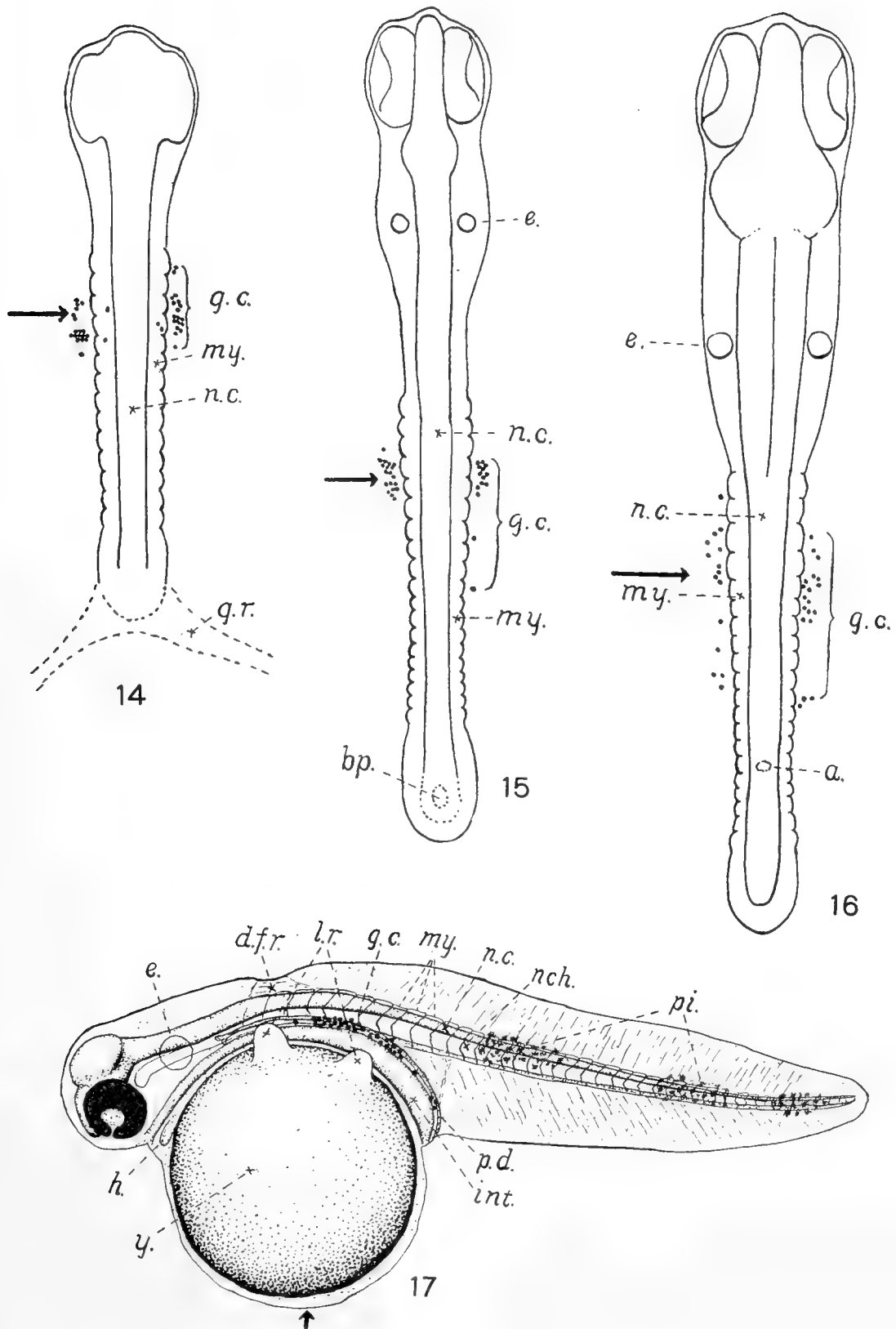
## EXPLANATION OF FIGURES

10. Cross section of embryo of stage 13.  $\times 110$ .
11. Cross section of embryo of stage 14.  $\times 110$ .
12. Outline sketch of right side of embryo of stage 6 as viewed from above.  $\times 45$ .
13. Outline sketch of embryo of stage 7, showing number and distribution of germ-cells.  $\times 45$ .



## EXPLANATION OF FIGURES

14. Outline sketch of embryo of stage 8, showing number and distribution of germ-cells.  $\times 45$ .
15. Outline sketch of embryo of stage 10, showing number and distribution of germ-cells.  $\times 45$ .
16. Outline sketch of embryo of stage 12, showing number and distribution of germ-cells.  $\times 45$ .
17. Side view of embryo of stage 13, showing number and distribution of germ-cells.  $\times 24$ .



## EXPLANATION OF FIGURES

18. Side view of anterior portion of embryo of stage 14, showing number and grouping of germ-cells.  $\times 24$ .

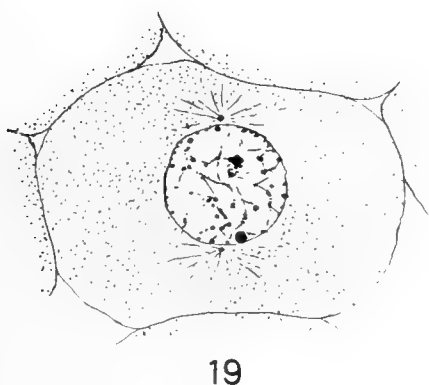
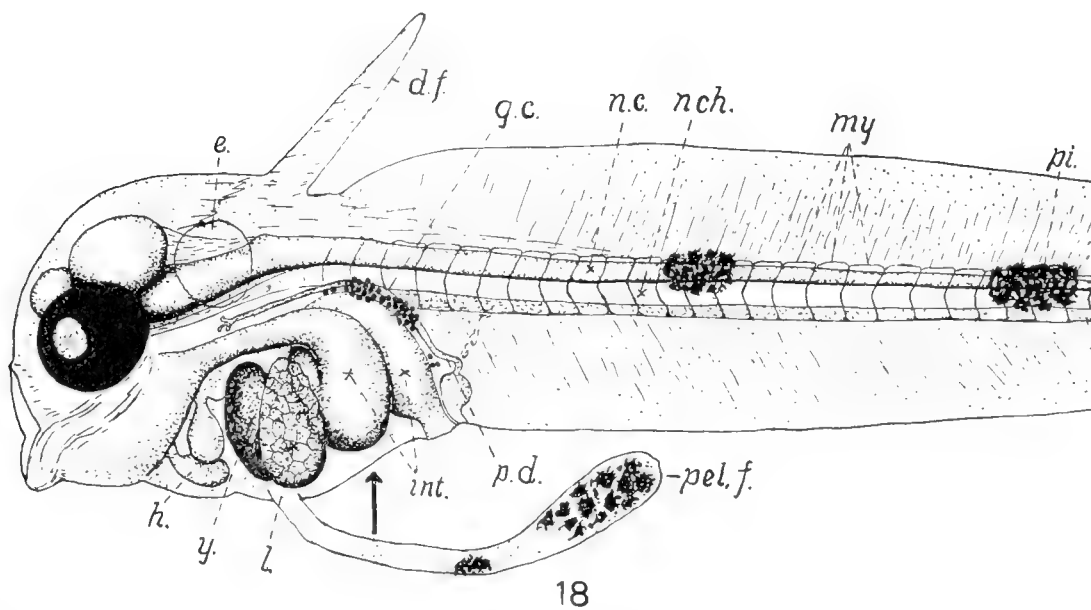
19 to 34 are drawn to the same scale and were outlined with the aid of a camera lucida. The details of the nucleus are in some cases reconstructed from two adjacent sections.

19. A single cell from embryo 1 A, as a type of cells of this early stage.  $\times 1260$ .

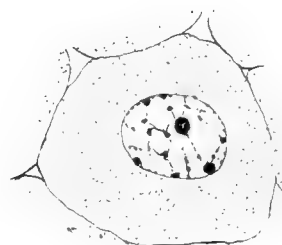
20. A single cell from embryo 2 A, a little older than the preceding.  $\times 1260$ .

21. From embryo 6 F. The earliest recognized germ-cell and some surrounding cells of the primary entoblast.  $\times 1260$ .

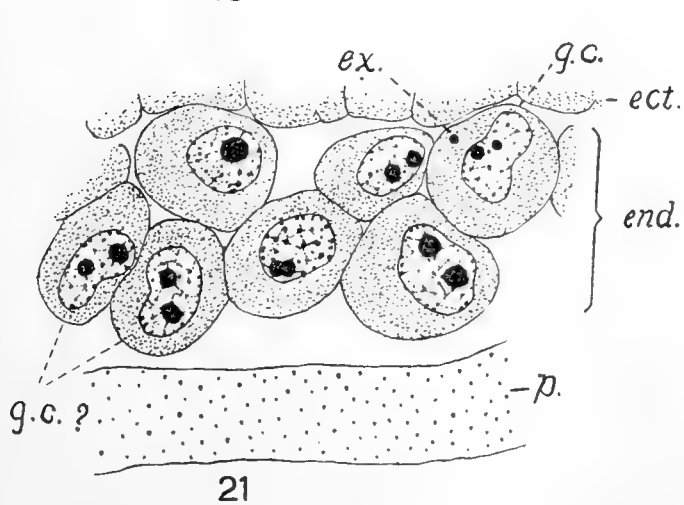
22. From embryo 6 D, just a little older than 6 F. This germ-cell lies in the early mesoblast.  $\times 1260$ .



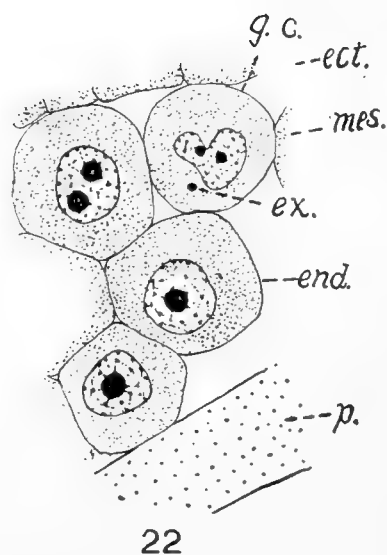
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## EXPLANATION OF FIGURES

23. Two germ-cells from embryo 6 E with some surrounding cells of the entoblast.  $\times 1260$ .

24. Germ-cells from embryo 7 Fr. Two plasmosomes of the reduced size appear within the nucleus, and another small deeply staining body, which may possibly be a fragment of plasmosome material about to be extruded.  $\times 1260$ .

25. Germ-cell from embryo 7 Fr. Shows a stage in plasmosome reduction. The nucleus contains one unreduced plasmosome, and one of reduced size. Opposite this one, in the cytoplasm is a mass recently separated from it.  $\times 1260$ .

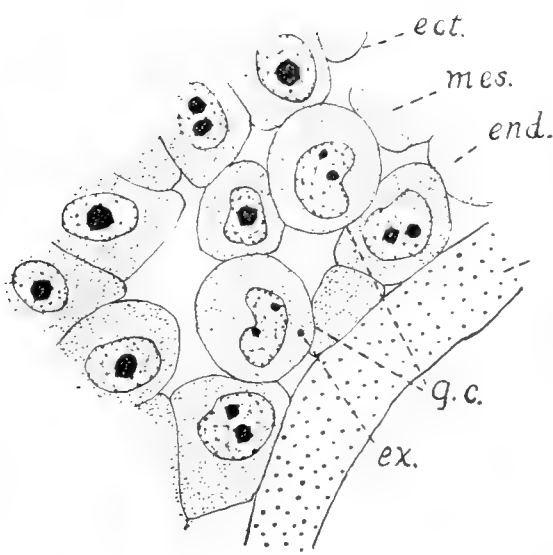
26. Germ-cell from embryo 7 Fr. A cell which has just completed plasmosome reduction. The nucleus contains two small plasmosomes. One is clearly in contact with the nuclear membrane and just opposite it a mass just separated from it.  $\times 1260$ .

27. Two germ-cells from embryo 7 Fr. These have both completed plasmosome reduction. In one of them the extruded plasmosome has separated into two parts.  $\times 1260$ .

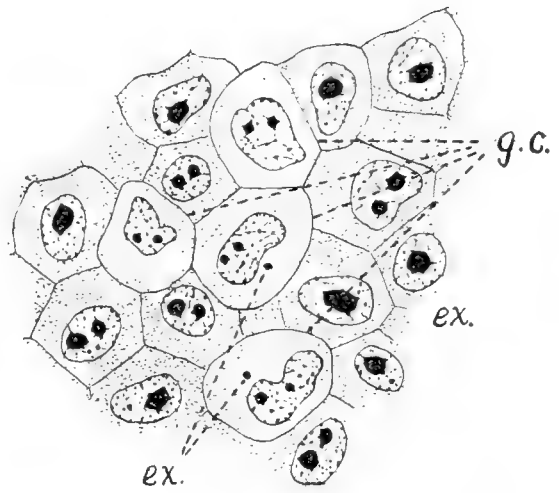
28. Four germ-cells from embryo 8 E. All of these lie in the solid lateral plate. Two of them show the extruded plasmosome in the cytoplasm of the cell.  $\times 1260$ .

29. A single germ-cell from embryo 9 A, lying in the splanchnic mesoblast. In this and in older embryos, the pigment cells are a conspicuous feature.  $\times 1260$ .

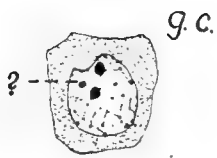
30. Two germ-cells from embryo 10 D, migrating from splanchnic to somatic layers of mesoblast.  $\times 1260$ .



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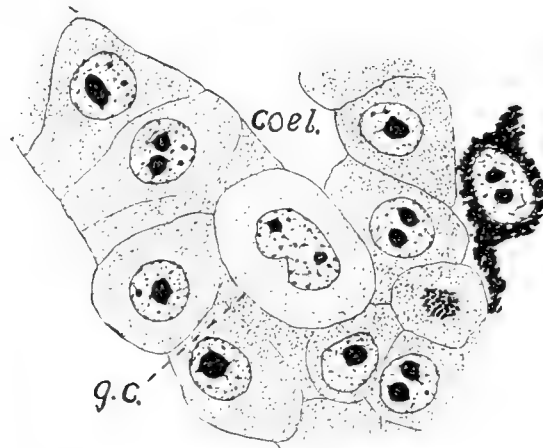
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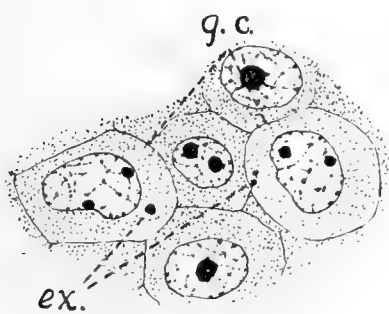
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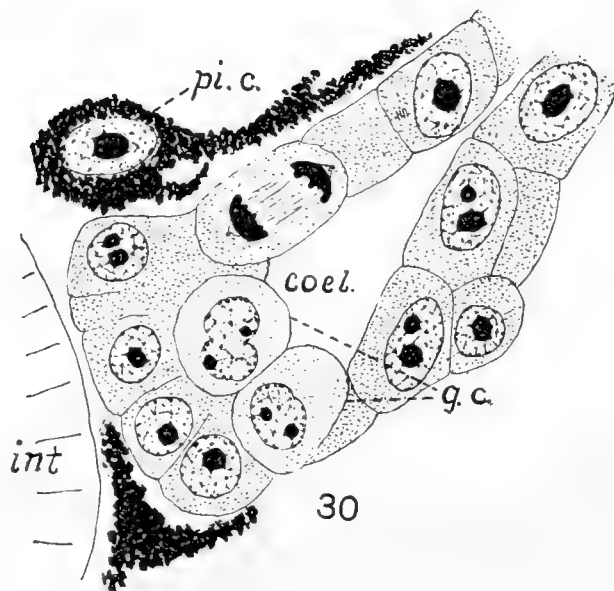
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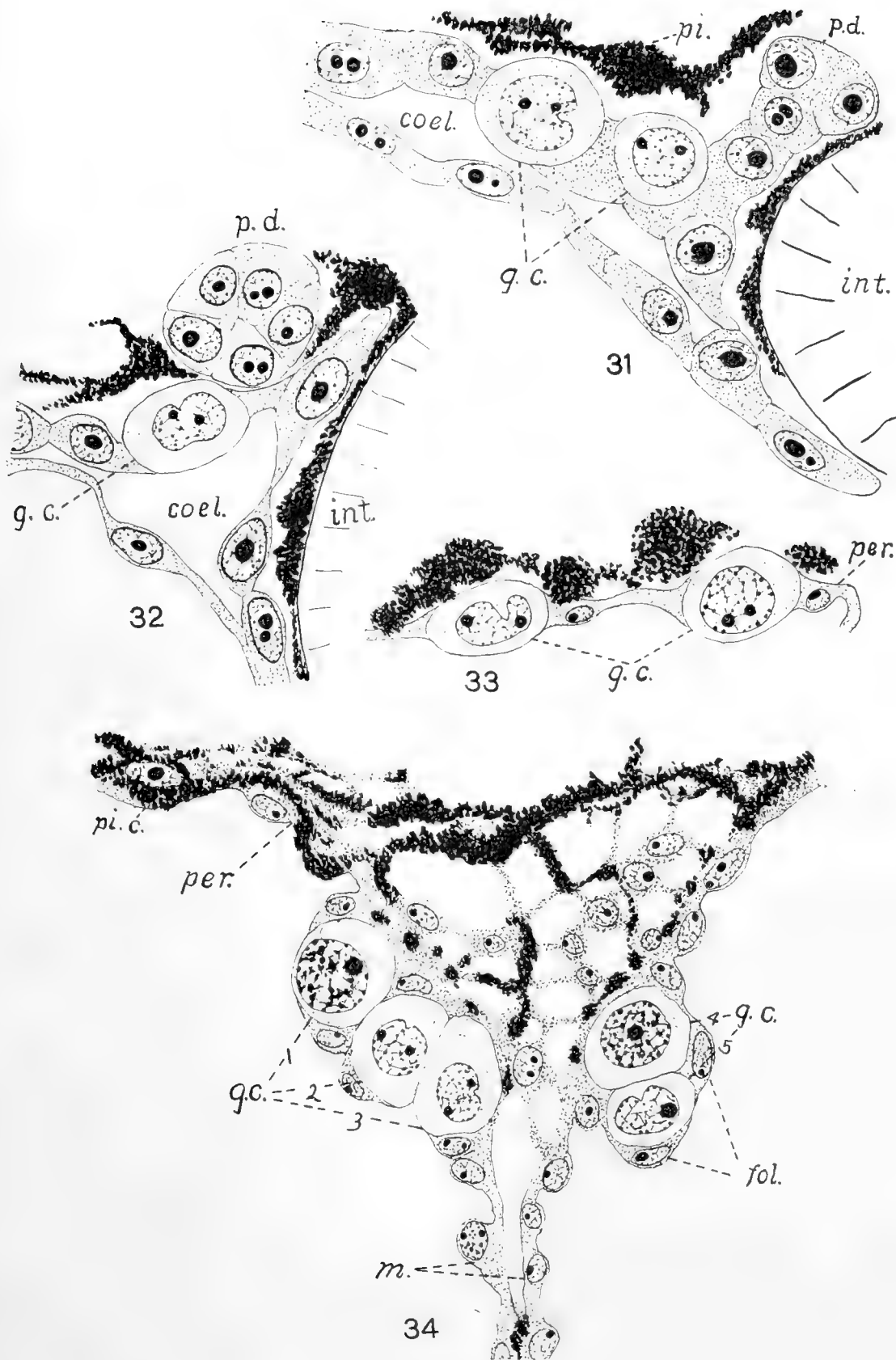
## EXPLANATION OF FIGURES

31. Two germ-cells from embryo 11 D. They have reached the somatic mesoblast and the pronephric duct is in process of formation.  $\times 1260$ .

32. A germ-cell from embryo 12 G. The pronephric duct has become separated from the coelom.  $\times 1260$ .

33. Two germ-cells from embryo 13 H. They lie in the dorsal peritoneal wall which had become very thin, leaving the large germ-cells standing out prominently.  $\times 1260$ .

34. A group of five germ-cells from embryo 14 C. They are massed at the base of the mesentery and are seen to be surrounded by flattened cells, the future follicle cells. The nuclei of three of these germ-cells show the changes which begin at this time.  $\times 1260$ .





# THE LATERAL WALL OF THE CAVUM NASI IN MAN, WITH ESPECIAL REFERENCE TO THE VARIOUS DEVELOPMENTAL STAGES

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FIFTY FIGURES

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## I INTRODUCTION

A survey of the literature on the lateral wall of the *cavum nasi* indicates that commendable researches have been carried out by different investigators. Although much careful work has been done, conflicting opinions are held on some points of the development and the gross anatomy of this portion of the olfactory organ. The idea of an unvarying typical form in the gross anatomy of the structures and relations in this region of the nose seems too much in evidence in some of the general textbooks of anatomy. In fact the exceptions in some instances to the descriptions given are so numerous that the exception is described rather than the average condition. This is especially true in the manner of communication of the nasofrontal duct with the middle nasal meatus. The ostium maxillare accessorium—a very common aperture indeed—does not receive the recognition it should have. According to my series of specimens, 62.5 per cent of ethmoidal regions possess in the adult three conchae. Books generally describe and picture two ethmoidal conchae as the typical number, and apparently would have us think that three ethmoidal conchae are rather exceptional.

In order to determine whether the embryology would account for the varied adult conditions one meets, I undertook the study of the various developmental stages in the formation of the complex lateral nasal wall. It was deemed essential that the embryological stages in the formation of the nasal cavity be considered before taking up for detailed study its lateral wall. The paper will, therefore, include: (1) a brief description of the developmental stages of the nasal fossae; (2) the detailed embryology of the lateral walls of the nasal fossae; (3) the gross anatomy of the lateral walls as presented in the term fetus and the young child; (4) the adult lateral walls with especial reference to some later developmental changes.

It is obvious that the fundamental structures to be considered in a study of the development of the lateral wall of the *cavum nasi*

are: (1) the nasal meatuses and the nasal conchae; (2) the accessory furrows and the accessory folds (conchae); (3) the anlagen of the sinus paranasales.

The materials used in this investigation include the following:

- a.* Human embryos at successive ages from 21 days up to the fetus at term;
- b.* Fifty lateral nasal walls of new-born children;
- c.* Twenty lateral nasal walls of children, ranging in age from birth to 15 years;
- d.* One hundred and fifty adult lateral nasal walls, ranging in age from 15 to 88 years;
- e.* Embryological and adult specimens of the lateral nasal wall of the cat, dog, muskrat, woodchuck, skunk, monkey, pig, sheep, and cow.

The lateral wall of the nasal cavity of human embryos, aged respectively 35, 43, 49, 58, 70, 83, 103, 120, 210, and 280 days, was modeled by the blotting-paper method. Some of the models were sectioned at appropriate planes to facilitate a more detailed study of parts. In a number of instances the nasal fossæ were also reconstructed, in order to aid in a better understanding of certain developmental stages. Through the kindness of Mrs. Gage, who had previously modeled a 21-day human embryo, I had the privilege of studying this region at this early stage of development.

The ages of all the human embryos studied were determined by the Mall-method (Catalogue of the collection of human embryos in the anatomical laboratory of the Johns Hopkins University, Baltimore, 1904).

I wish to take this opportunity for expressing grateful acknowledgment to Professors Kerr, Kingsbury, and Tinker for helpful suggestions. I also wish to express my appreciation of the abundant material and other facilities placed at my disposal by the departments of Anatomy, and Embryology and Histology. To Professor and Mrs. Gage, for many courtesies extended during this piece of research, I wish to express thanks.

## II THE FORMATION OF THE NASAL FOSSAE

*The nasal anlage*

The nasal anlage apparently establishes itself about the third week of embryonal life as localized thickenings of the ectoderm. Kallius and Mihalkovics place the earliest trace of the nasal anlage at the beginning of the third week, and Bryce at the end of the third week of embryonal life. Mrs. Gage refers to the nasal epithelium in a "three weeks human embryo."

These thickened ectodermal or nasal areas are situated on both sides of the outer surface of the wall of the fore-brain, just superior to the primitive oral fossa. Whether the nasal areas of the human embryo are at first connected, and later undergo division into two distinctly separate halves, is not definitely known. The H-shaped nasal area of Mrs. Gage's 21-day embryo seems significant. She figures two lateral plates connected by an intervening bar of apparently similarly thickened ectoderm. Minot says: "It is possible that more exact observation will show that in all vertebrates there is at first a single plate, which is early divided." Doubtless this will remain a mooted point in man until a sufficient number of human embryos of the proper age fall into the hands of different observers.

Bedford found the thickness of the olfactory areas in swine embryos of 5 mm. length to be 0.075 mm. Kallius found that, while the general ectodermal thickness was 0.01 mm., the thickened nasal areas measured from 0.044 to 0.060 mm.

*The nasal pits*

During the fourth week the nasal areas become depressed—according to Mihalkovics at the end of the third week. This depression is not due to an invagination of the nasal areas, but it is passive and is caused by an increase in the thickness of the surrounding mesoderm. These ridges of thickened mesoderm around the nasal areas push the ectoderm into relief. In this manner each nasal area becomes surrounded by a fold; and is

thus passively depressed. The folds are not complete but are deficient inferiorly, and the thickening is most marked medially and laterally. The comparatively excessive medial and lateral thickening is thought by Kallius to be in anticipation of the later medial and lateral nasal processes. The nasal plates or areas thus become the olfactory or nasal pits, separated by a broad mass of tissue—the fronto-nasal process or the Stirnfortsatz of German embryologists.

The pits are primitively more or less pyriform in shape. The clubbed ends are directed somewhat medially and towards the vertex of the head, and the inferior extremities are directed somewhat laterally and towards the primitive oral fossa—the stomodæum. As the pits deepen they seem to separate the inferior portion of the fronto-nasal process on both sides into medial and lateral parts—the anlagen of the medial and the lateral nasal processes. During the latter part of the fourth week, or early in the fifth week, the median portion of the fronto-nasal process undergoes further differentiation into a median or unpaired part, and two lateral or paired parts. The latter are globe-like and will be spoken of as the medial nasal processes (*Processi globulares*, His), and they form the immediate medial boundaries of the nasal pits. At the same time the lateral portions of the fronto-nasal projection grow caudally and form the lateral nasal processes—the immediate lateral boundaries of the nasal pits, or the *primitive* lateral nasal walls.

Up to this time the nasal pits are not closed in inferiorly, but communicate freely with the primitive oral fossa. In other words, the nasal pits and the oral fossa represent in a sense a common cavity. At this stage of development the maxillary processes of the first or mandibular arches grow ventrally and medially and abut—later fuse—with the medial nasal processes (*Processi globulares*). This fusion closes in the superior boundary of the primitive oral cleft, and at the same time shuts off the path of communication between the nasal pits and the oral cavity, i.e., the nasal pits are closed in inferiorly (figs. 1 and 3). The coalescence of the maxillary processes with the medial nasal processes forms the primitive inferior boundaries of the nasal pits; subse-



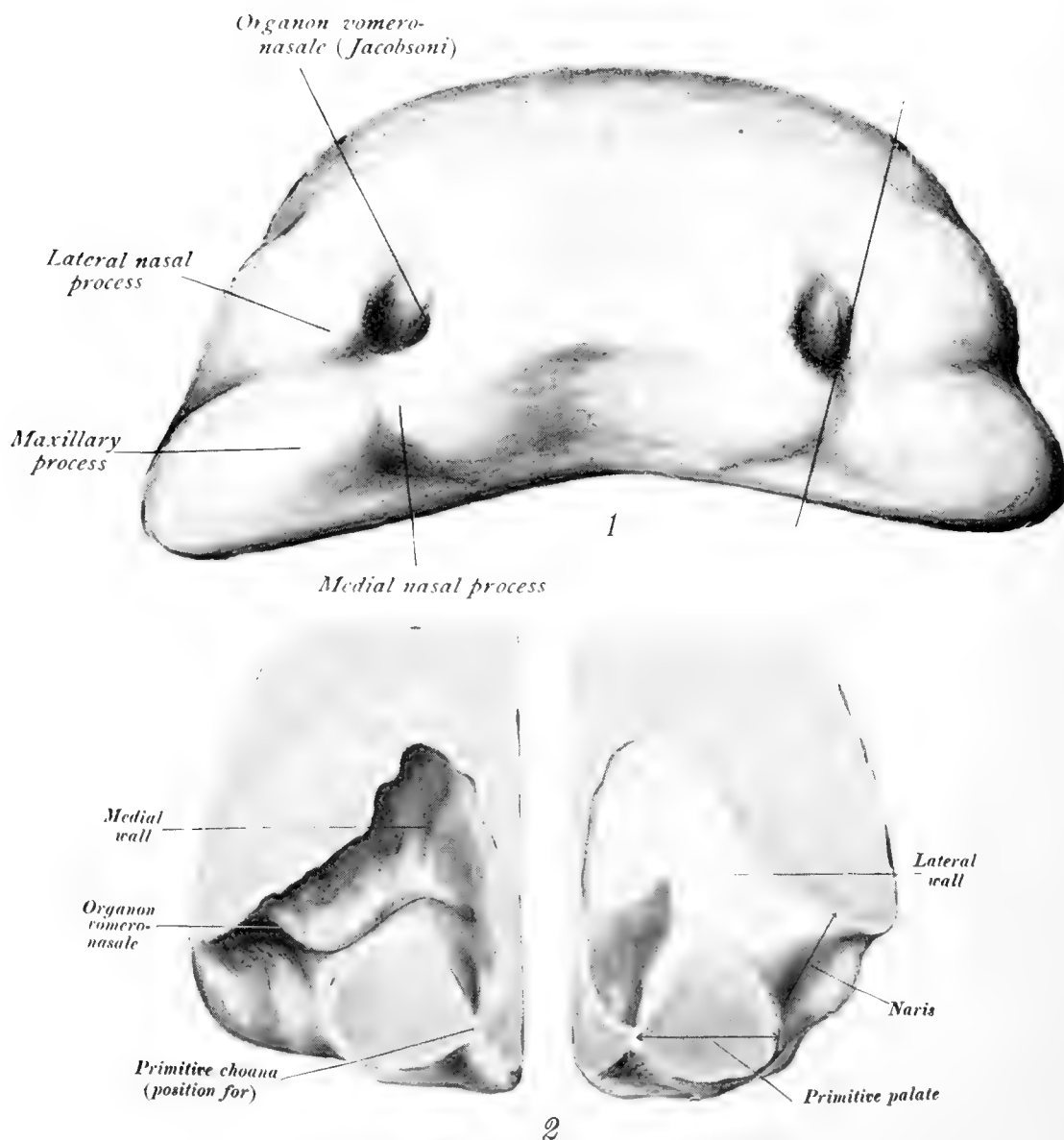
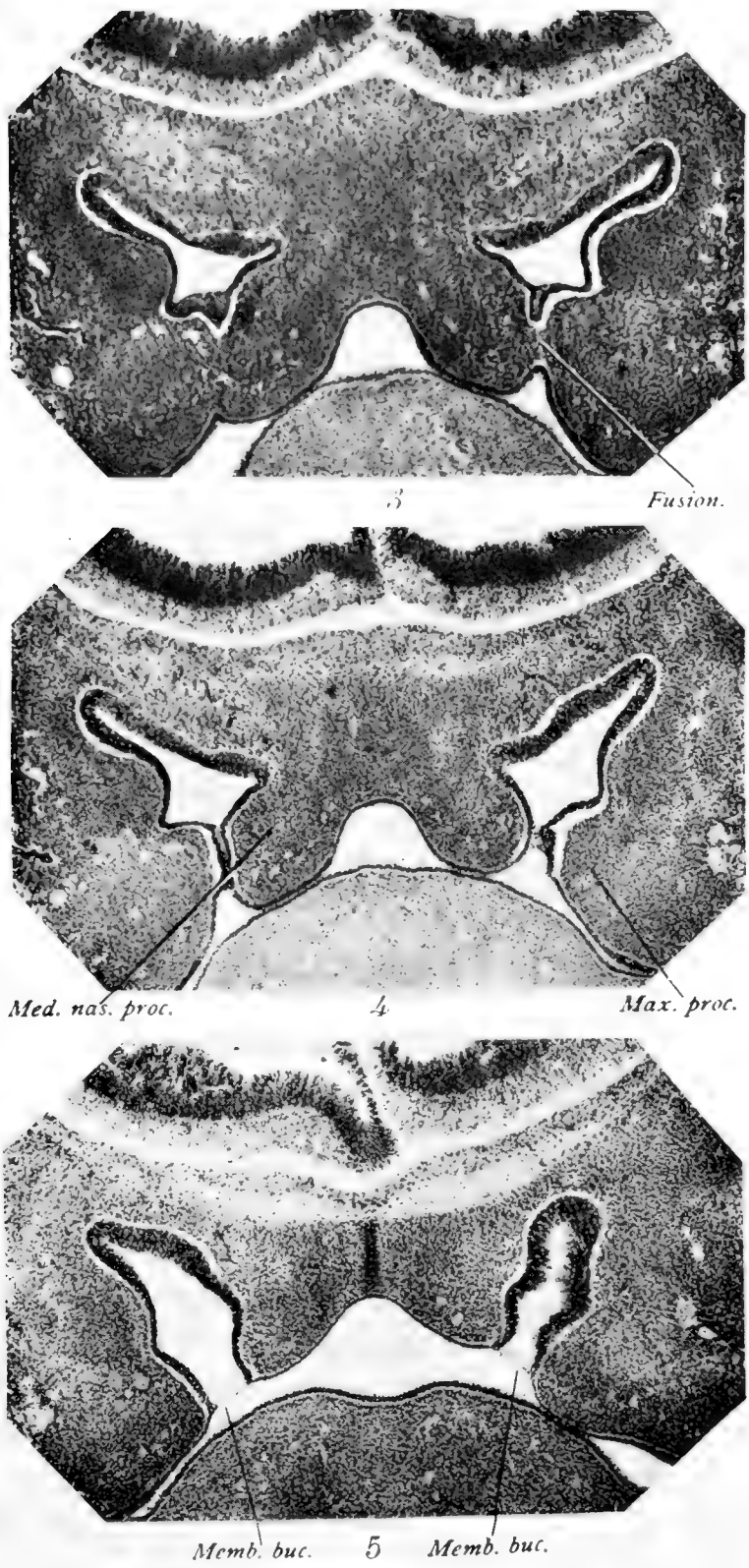
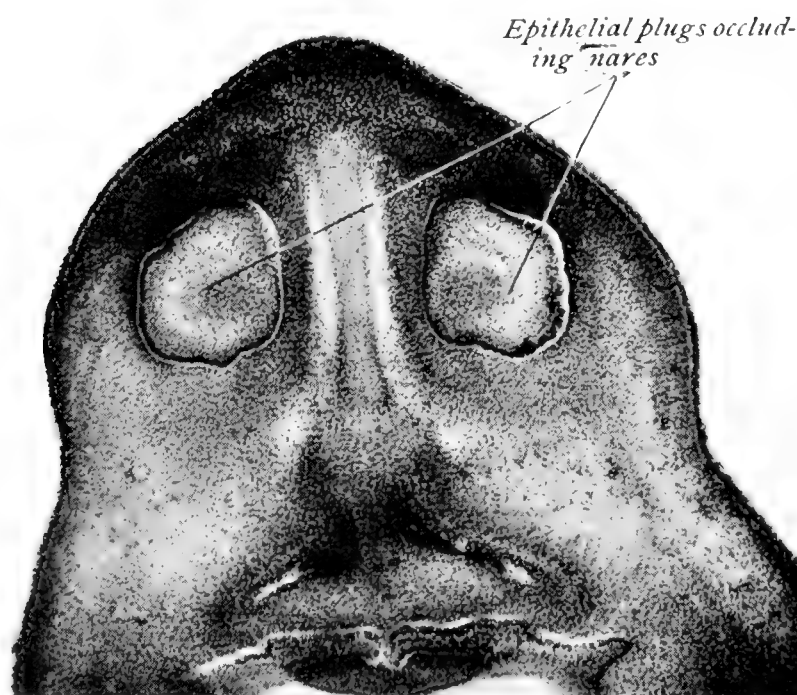


FIG. 1 ( $\times 30$ ) Drawing of a reconstruction of portion of the head of an embryo aged approximately 35 days (Human embryo, No. 6, Cornell University series, slides 50-57 inclusive). The figure represents a caudo-ventral view of the reconstruction.

Note the wide separation of the nasal fossae at this time, and that the fossae do not communicate with the mouth cavity. The maxillary and the medial nasal processes have fused, thus shutting off the nasal pits from the mouth cavity. The lateral nasal processes have not yet fused with the medial nasal processes. The external nose has not yet taken on any definite shape. Note the points for the primitive choanae and the extent of the primitive palate.

FIG. 2 ( $\times 45$ ) Drawing of a reconstruction of the left nasal fossa. The reconstruction is from the same embryo as that in fig. 1, and the plane of section is indicated by the line drawn over the left nasal fossa in fig. 1. Note the very simple lateral nasal wall at this stage of development and compare it with the medial wall. In a short time the lateral wall becomes much more complex and the medial wall relatively less so.





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## EXPLANATION OF FIGURES

FIGS. 3, 4, 5, (33.2) Photomicrographs of frontal sections through the head of an embryo aged approximately 35 days (Human embryo, No. 26, Cornell University series, slides 19 and 20). The sections are in the region of the primitive nasal fossae. Section fig. 3 is the most ventral and section fig. 5 the most dorsal of the three.

FIG. 3. Note that in the region of the coalescence of the medial nasal and the maxillary processes there is no intervening ectoderm. Fusion is complete and absolute, in that the mesenchymal tissue of the maxillary and medial nasal processes is continuous.

FIG. 4. Note the ectodermal strands between the medial nasal and the maxillary processes; the mesenchymal tissue of these processes is not continuous. Compare this condition with fig. 3.

FIG. 5. On both sides the two layers of epithelium, oral and nasal, have become attenuated and thinned out to represent single layers of cells. These are the bucconasal membranes which ultimately rupture to establish the primitive choanae.

*Fusion*, = coalescence between the maxillary and the medial nasal processes; *Med. nas. proc.*, = medial nasal process; *Max. proc.*, = maxillary process; *Memb. buc.*, = membrana bucconasalis.

FIG. 6 (× 18) Photomicrograph of a frontal section through the ventral portion of the nose of an embryo aged approximately 49 days (Human embryo, No. 28, Cornell University series, slide 40).

Shows the epithelial plugs occluding the nares. This is a rather common condition during the latter part of the second month and throughout the third month. It also occurs after this period, as I have seen it in a 120 day-embryo.

quently, however, the later extensions of the lateral nasal processes, medially and ventrally above the maxillary processes, meet and fuse with the medial nasal processes, to form the immediate inferior boundaries of the nasal pits (fig. 1). His has also pointed out, in case of arrested development in this region, that at times the lateral and medial nasal processes fuse, but that the maxillary process, on one or both sides, remains short and does not develop sufficiently to fuse with the medial nasal process as it normally does. In such cases the nasal pits are closed in below and are separated from the oral cavity, yet clefts exist between the medial nasal and the maxillary processes.

Fusion also takes place laterally between the maxillary and lateral nasal processes, and from the strands of ectodermal tissue caught between the edges of the coalescing processes, the nasolacrimal ducts develop, *i.e.*, the ectodermal strands acquire lumina. These ducts will again be referred to in connection with their apertures on the lateral nasal walls. The ducts usually establish communications with the inferior nasal meatuses at about *term*. This may however be longer delayed.

For some time the lines of fusion of the maxillary and the lateral nasal processes with the medial nasal processes are represented by strands of ectodermal tissue. These ectodermal fusion-lines soon disappear ventrally and are replaced by indifferent mesenchyme, *i.e.*, the mesenchymal tissue of the maxillary and the lateral nasal processes becomes continuous with that of the medial nasal processes. Fusion in this manner becomes permanent and absolute (fig. 3). Farther dorsally the ectodermal tissue does not wholly disappear, but strands of the latter tissue remain between the abutting processes. In these positions the primitive choanæ ultimately become established (figs. 2 and 4).

#### *The primitive nasal fossae*

By this time (35-day embryo) the nasal pits have deepened dorsally, and more or less superiorly and inferiorly. The olfactory organ is now represented by two blind pouches lying in the mesenchymal tissue above the oral cavity. The pits have now

developed sufficiently so that they may be termed the primitive nasal fossae. The fossae communicate freely with the exterior by means of the nares, but, in the absence of choanæ, they end blindly at their dorsal and inferior terminations (fig. 2). They are rather widely separated at this time (fig. 1). A reference to fig. 2 will show both the lateral and medial walls of the left nasal fossa of a 35-day embryo. It will be noticed that the lateral wall is extremely simple, presenting merely a plane surface. The medial or septal wall is slightly grooved and the groove overhung by a fold. This is the anlage of the organon vomeronasale (Jacobsoni). Note also that the fossa ends blindly and that there is no connection between the oral and the nasal fossa at this time. The point of the primitive choana (posterior naris) is nearly thinned out sufficiently to represent the membrana buconasalis (fig. 5).

#### *The primitive choanæ*

The dorsal extension of the blind pouches or primitive nasal fossae continues until the ectoderm of the nasal fossae meets the ectoderm of the oral fossa. We have now in these positions merely thin membranes composed of two layers of abutting epithelium—nasal and oral—separating the dorsal portions of the primitive nasal fossae from the oral cavity. These membranes, which have been carefully studied and named by Hochstetter, the “*Membranae buconasales*,” (fig. 5) become so attenuated and thinned out that they finally rupture. The membranes may rupture at the same time or each may rupture independently of its mate.

According to the embryos studied for the substance of this paper the buconasal membranes rupture from the 35th to the 38th day of embryonal life. Sudler found connection between the oral and nasal fossae in a 5-weeks embryo. Hochstetter found in an embryo of “11 mm. lang bei 9 mm. Kopflänge” that the nasal fossae ended blindly dorsally, and in an embryo of “15.5 mm. Länge und 10 mm. Kopflänge,” the buconasal membranes were broken through. He, however, found that the membrane was rather excessive on one side, apparently indicating recent rupture. The approximate age of these embryos would be 33 and 39 days,

respectively. Kölliker places the time of establishment of the primitive choanæ "in der zweiten Hälfte des zweiten Monates." The apparent discrepancy in the time at which the bucconasal membranes rupture is doubtless due to different methods of determining the age of embryos.

As a result of the rupture of the bucconasal membranes we have established two openings—the primitive choanae (primitive Gaumenspalten, Dursy; innere Nasengänge, innere Nasenlöcher, Kölliker). There is thus established in man for a second time a communication between the oral and the nasal fossae. The hitherto blindly ending nasal fossae now communicate not only with the exterior by means of the nares, but also with the mouth cavity by means of the primitive choanæ.

#### *Epithelial plugs in the nares*

When first formed the nares communicate freely with the exterior, but shortly afterwards, say about the fortieth day of embryonal life, the lumina of the nares in very many cases become filled in with epithelial plugs (figs. 6 and 7). This plugging which is due to a proliferation of the epithelial cells, seems to take place some time after the fusion of the medial and lateral nasal processes. In some cases the plugging is absolute, and in others deviating passages through the plugs may be seen. Sometimes the plugs are more or less fenestrated. I find plugging rather frequent from the fortieth to the sixtieth day. I have, however, seen it in a 120-day embryo. The passages (nares) are apparently later opened by a shedding of the epithelial plugs rather than by a resorption of the cells—probably both factors are involved. As evidence of a shedding of the epithelial plugs we frequently find shreds of epithelial masses extruding from the nares.

#### *The primitive palate*

With the formation of the primitive choanae we have also established the primitive palate, *i.e.*, the portion of the roof of the oral fossa extending from the primitive choanae to the nares.

This condition is, however, of short duration because the definitive palate soon begins to form, and the lateral walls of the nasal fossae thus become limited inferiorly. In the formation of the definitive palate the nasal fossae appropriate a considerable portion of the primitive oral cavity. This is readily understood when we recall the superior boundary of the primitive oral cavity dorsally. Ventrally the mouth cavity is bounded superiorly by the primitive palate, but dorsally the mouth cavity extends into the future nasal cavity until the formation of the definitive palate establishes a roof for the mouth cavity dorsally (figs. 8, 9, 11, 13, 14, and 15).

*The formation of the definitive palate*

A reference to figs. 10, 13, 14, and 15 will indicate the stages in the formation of the definitive palate, and the manner in which the lateral nasal wall becomes limited inferiorly. The first step in the production of the definitive palate is the appearance of the palatal ridges. These are more or less wedge-shaped processes which grow caudally and somewhat medially from the medial sides of the maxillary processes. The palatal processes appear from the forty-fifth to the forty-eighth day of embryonal life, and at first hang almost vertically towards the mouth cavity, on either side of the tongue (fig. 13). They extend from the line of union between the medial nasal and the maxillary processes, where they are continuous with the primitive palate, dorsally to the wall of the pharynx, where they are continuous with the palato-pharyngeal folds. The palatal processes limit the lateral walls of the cavum nasi inferiorly.

It will be noticed that the tongue is at first between the palatal processes, *i.e.*, the processes extend below the level of the dorsum of the tongue (figs. 10 and 11). Soon the tongue sinks and comes to occupy a lower position in the mouth cavity. With this change of the tongue the palatal processes become rotated from an almost vertical and sagittal plane to a horizontal plane (compare figs. 13 and 14). The processes now shortly meet in the median plane



## EXPLANATION OF FIGURES

FIGS. 7 to 12 ( $\times 10.5$ ) Photomicrographs of frontal sections of the head of an embryo aged about 43 days (Human embryo, No. 3, Cornell University series, sections 406, 380, 365, 360, 350, 325).

Note the plugging of the nares in fig. 7, and that the conchal anlagen have no cartilage in them at this age (figs. 8-11). The mesenchymal tissue is, however, already undergoing condensation in anticipation of cartilage in the regions of the nasal septum and the lateral nasal walls. Compare this condition or pre-cartilage stage with figs. 16 to 20, and with figs. 32 to 38. Note the relation of the tongue to the palatal processes in figs. 8 to 12.

The section shown in fig. 7 is the farthest ventral and that shown in fig. 12 the farthest dorsal in the series.

*P. pal.*, = processus palatinus; *Eth. fold*, = ethmoidal fold; *Max. fold*, = maxillary fold.

FIG. 13 (16.6) Drawing of a reconstruction of the lateral wall of the nasal cavity of an embryo aged approximately 43 days (Human embryo, No. 3, Cornell University series, sections 300-420 inclusive).

Shows the vertical position of the palatal process at this time. The definitive palate is not yet formed. Note also the primitive concha inferior (*Max. fold*) and that it occupies the greater portion of the lateral wall at this early stage. The primitive ethmoidal fold (*Eth. fold*)—the anlage of the ethmoidal conchae—is very rudimentary at this time.

*Eth. fold*, = ethmoidal fold; *Max. fold*, = maxillary fold; *P. pal.*, = palatal process.

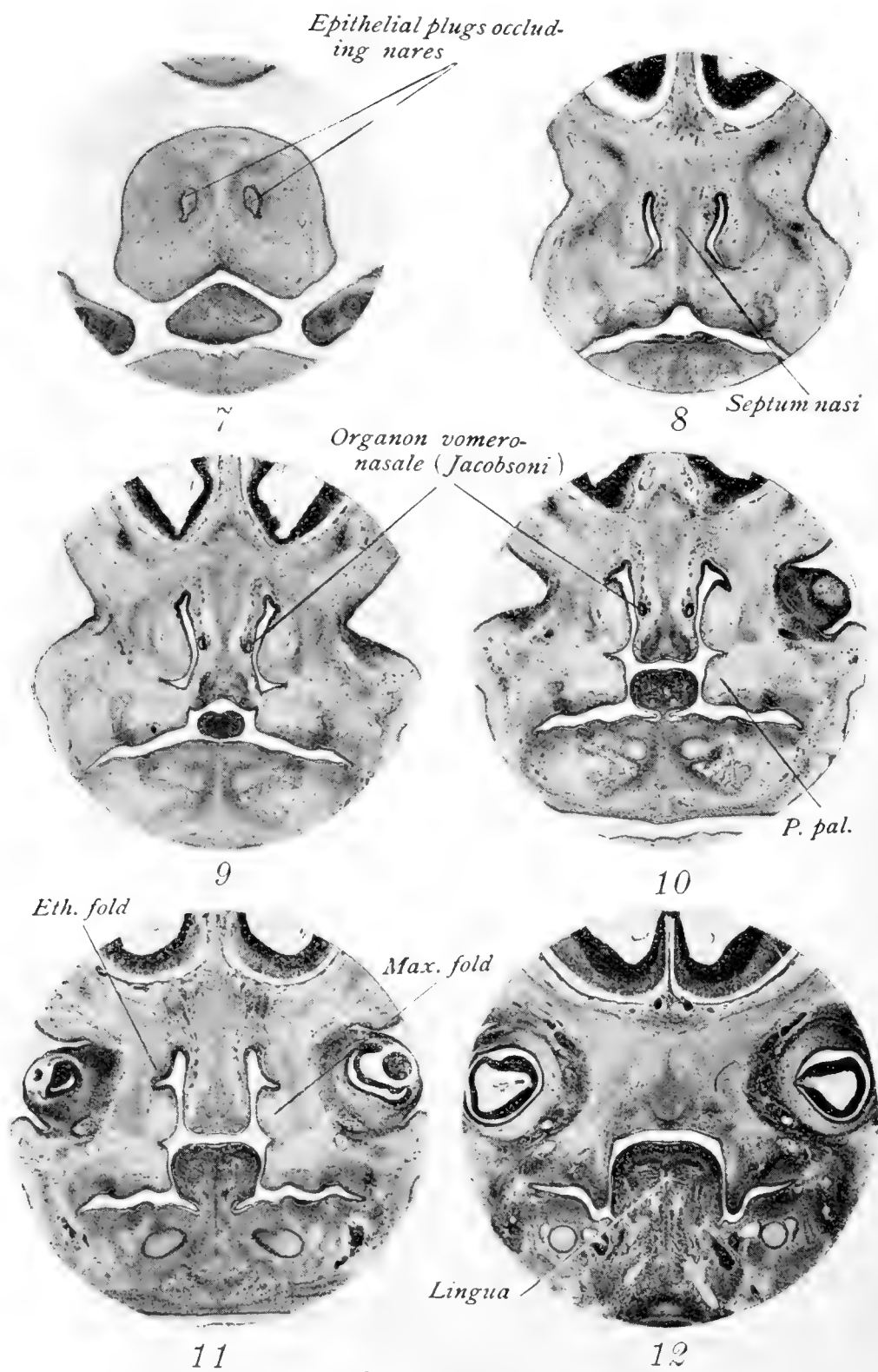
FIG. 14 ( $\times 16.6$ ) Drawing of a reconstruction of the lateral wall of the nasal cavity of an embryo aged approximately 49 days (Human embryo, No. 28, Cornell University series, slides 40-51 inclusive).

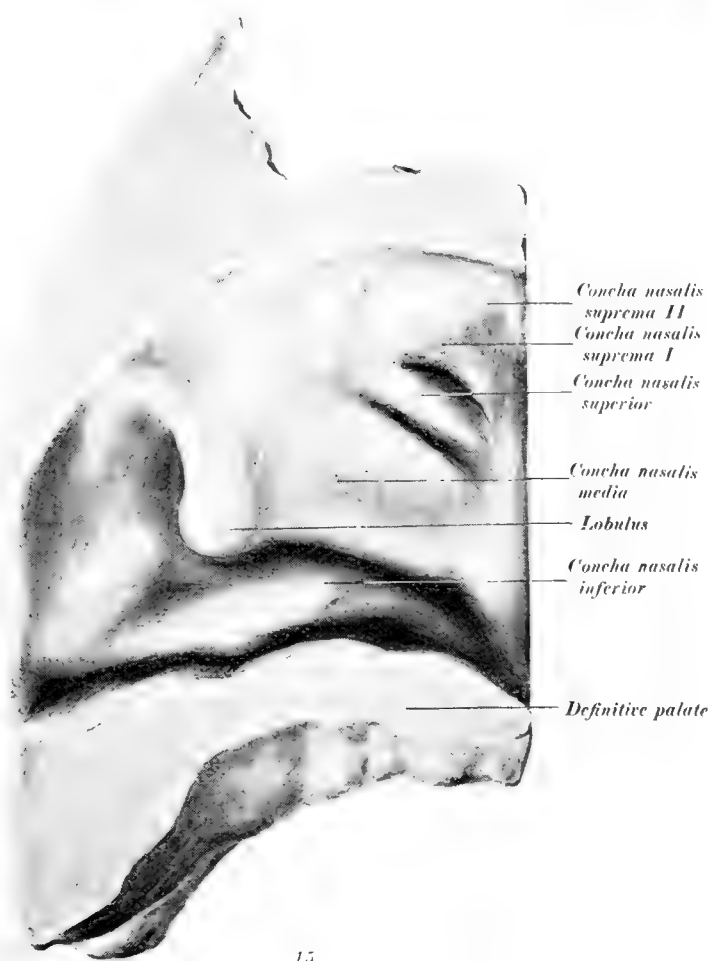
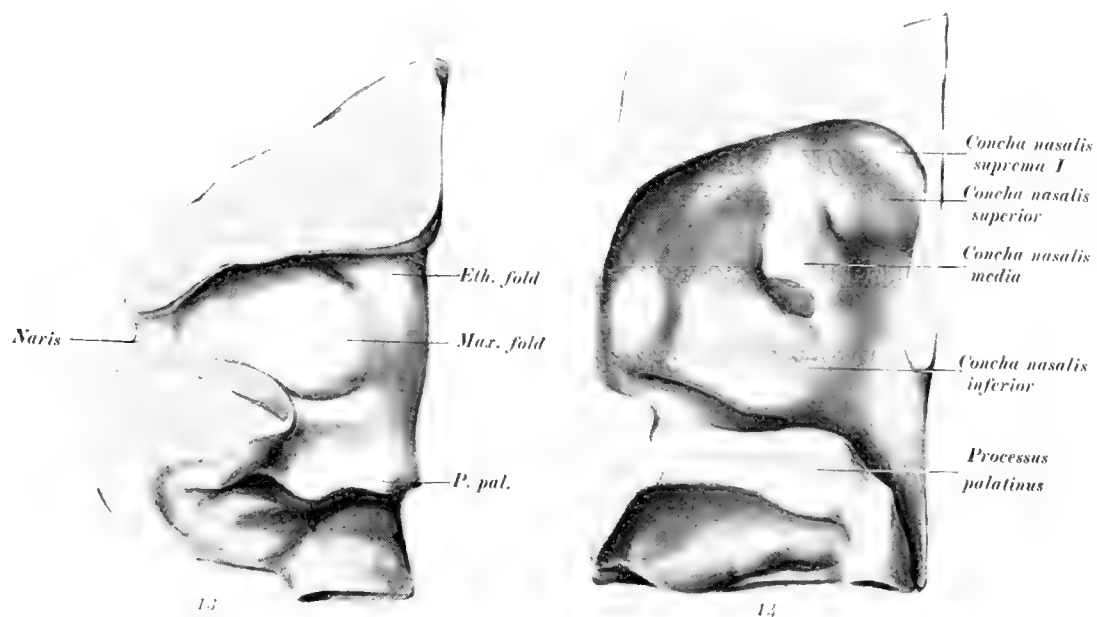
Compare horizontal position of the palatal process with the process in fig. 13. The ethmoidal region now has two folds well marked, and a rudimentary third presents in the superior and dorsal portion of the lateral wall. The concha nasalis inferior occupies comparatively less of the lateral wall. Ventrally it will be noticed that the reconstruction does not include all of the lateral wall. The ventral portion of the primitive palate is, therefore, not represented in the reconstruction.

FIG. 15 ( $\times 6.6$ ) Drawing of a reconstruction of portion of the right wall of the nasal cavity of an embryo aged about 105 days (Human embryo, No. 43, Cornell University series, slides 1-90 inclusive).

The definitive palate is completed and the choana has assumed the adult position. Compare this with figs. 2, 13, and 14. The ethmoidal region now presents four conchae, the highest of which is not yet well differentiated. Note the marked lobule in the region of the knee of the concha nasalis media.







above the tongue, and fuse from before backwards along the opposed edges. In this manner the separation of the nasal fossae from the mouth cavity is made complete and permanent. However in the ventral portion of the palate, where the palatal processes do not come in contact with each other (due to the interposition of the tissue separating the primitive choanae) the separation of the nasal cavity from the mouth cavity is not entirely complete. This is brought about by the medial nasal processes uniting and extending dorsally in the roof of the mouth cavity as the inter-maxillary process. The latter process projects farthest dorsally in the median plane, and on its lateral borders it is met by the ventral ends of the palatal processes. At these contact points the incisive canals are formed, and the latter at times serve as a means of permanent communication between the oral and nasal cavities. The lumina of the incisive canals are, however, generally obliterated early in life.

*The permanent choanae and the permanent nasal fossae*

Coincidentally with these changes in the formation of the nasal fossae, the primitive choanae elongate and ultimately occupy their definitive position, and thus become the permanent or secondary choanae. The changes in the formation of the permanent choanae also aid materially in increasing the ventrodorsal extent of the nasal cavity. The latter now begins to take on its adult form. The nasal septum which already has separated the nasal fossae ventrally, rapidly fuses with the palate in the median plane, *i.e.*, along the line of fusion of the palatal processes. In this manner the cavum nasi is divided into the nasal fossae. This division takes place from before backwards.

Before the first appearance of the palatal processes as inferior boundaries of the nasal fossae, the lateral walls of the fossae have begun in a simple manner to form the complex configuration which characterizes the adult lateral nasal walls.

### III THE DEVELOPMENT OF THE LATERAL WALL OF THE CAVUM NASI

In the preceding paragraphs we considered the successive stages in the establishment of the nasal cavity, from the thickening of the ectoderm to form the nasal anlage, to the formation of the nasal fossae. We noted the extreme simplicity of the lateral wall of the nasal cavity during the early stages of development. With these considerations on the various developmental stages of the nasal fossae and the early condition of the lateral wall as a basis, we may now to better advantage follow in detail the stages in the formation of the various structures found on the lateral wall. We will, therefore, pass, in order, from the very simple lateral wall of the early embryo to the complexly configured wall of the term fetus.

#### *The formation of conchal anlages*

A reference to fig. 2 will indicate the extremely simple lateral wall of the nasal fossa of a 35-day embryo. It will be noticed that there is no evidence of the later complexity and configuration caused by the nasal conchae, the nasal meatuses, the accessory conchae and furrows, and the anlages of the paranasal chambers. In fact the medial or septal wall is more complicated at this time than is the lateral wall—this standing in marked contrast to later conditions (compare figs. 2, 13, 14, and 21).

Some days later (38- to 40-day embryo) the inferior portion of the lateral wall immediately superior to the primitive palatal processes begins to bulge towards the lumen of the nasal fossa. In fact this bulging for a brief time occupies nearly the whole of the vertical portion of the wall, and approximately the dorsal two-thirds. This fold represents the anlage of the concha nasalis inferior (maxilloturbinal) (fig. 13). Shortly after this (40 to 43 days) a second fold appears superior and somewhat dorsal to the first one. The former (ethmoidal fold) is placed in the superior and dorsal angle of the lateral wall. It seems also to extend up on the nasal septum at this point, and some of its prominence is

really due to septal tissue. This fold is the anlage of the conchae nasales ethmoidales, and in position and relations represents the concha nasalis media (Ethmoturbinale I, Peter; zweite Hauptmuschel, Killian; untere Siebbeinmuschel, Zuckerkandl; Basoturbinale, Schönemann). The fold of the agger nasi (nasoturbinal) can hardly be said to be in evidence at this early stage. It appears later, ventral and superior to the concha nasalis inferior, and ventral to the concha nasalis media.

*Views as to the genesis of the primitive nasal folds and furrows*

From the above we gather that the lateral nasal wall is early thrown into ridges or folds which are bordered by furrows or depressions. Just how these primitive folds (primitive conchae) and furrows (primitive meatuses) of the lateral wall are formed is interpreted differently by various writers. While at first thought the views held seem widely divergent, they appear less so after we carefully analyze them. Some of the theories entertained are, however, without foundation.

It was at one time held by some observers that the folds were due to cartilaginous strands pushing the lateral nasal wall medially in the positions of the folds. This theory is, however, not tenable because the folds (primitive conchae) are invariably present some time before cartilage is found in them. Only later does some of the indifferent mesenchyme contained in the folds change into cartilage; therefore it can have no connection whatever with the establishment of the *primitive* folds (conchae).

Legal and Schönemann think that the folds are elevations left by excavations of furrows on the lateral nasal wall. Legal in offering this explanation especially referred to the inferior nasal concha. Later Schönemann concludes "dass nicht die Legalsche Angabe über die Bildung der unteren Muschel völlig zu Recht besteht, sondern dass auch für die sämtlichen anderen Muscheln ein ähnlicher Bildungsmodus angenommen werden muss."

Mihalkovics, according to Schönemann, holds that the folds are at first "frei vorwachsende Duplikaturen der Schleimhaut." Glas, who investigated this field in rats, comes to the following

conclusion: "Der Bildungsmodus der Muscheln ist die Resultierende zweier Komponenten: (1) des Auswachsens in die Wandpartien einwachsender Epithelleisten (Fissuren), (2) des Vorwachsens bestimmter Wandpartien."

After a study of these early conditions I am led to believe that the first change in the lateral nasal wall from a more or less even surface (fig. 2) is the production of very shallow grooves, the latter appearing inferior and superior to the *position* of the primitive concha inferior. These shallow grooves at once throw into slight relief the greater portion of the lateral nasal wall—the anlage of the concha nasalis inferior (maxilloturbinal). I believe that the mesenchymal tissue contained within this primitive fold almost simultaneously undergoes proliferation, thus aiding in early making the fold more prominent. The mucous membrane over the fold may also become thickened, but according to my observations this thickening for the primitive concha inferior is slight. I cannot agree that the formation of a well marked maxillary fold or primitive concha nasalis inferior is wholly due to the formation and deepening of the bordering furrows but, as stated before, I believe that the formation of very shallow grooves is the primitive step in conchal formation. The proliferation of the mesenchyme (probably also the mucous membrane to some extent) aids materially in causing the primitive concha inferior to bulge into the lumen of the nasal fossa, and the bordering furrows to become passively deeper (figs. 10 and 11).

The ethmoidal fold appears next in the extreme dorsal and superior portion of the nasal fossa, in the angle formed by the lateral wall and the nasal septum in this position (figs. 11 and 13). Since the primitive concha inferior (maxillary fold) takes up the greater portion of the lateral wall at this time there is little room for the primitive ethmoidal fold (fig. 13). For this reason the latter fold arises in part from the septum. Later the nasal fossa increases in the dorsosuperior direction, and with this the primitive ethmoidal fold passively migrates from the septum. The mucous membrane over the ethmoidal fold is as a rule thickened, and this thickening causes the primitive fold to show up well in

frontal sections (fig. 11). This, however, does not detract from the statement that the groove between the ethmoidal and maxillary folds is present, at least to some extent, before the folds have gained any appreciable bulging. Later we have a proliferation of the mesenchyme and a deepening of the furrow (primitive meatus nasi medius) inferior to it, hence the ethmoidal fold becomes more prominent. Gradually in place of the single ethmoidal fold we have, with the increase of the nasal fossa in the dorso-superior direction, the establishment of anlagen of the individual ethmoidal conchae—this, as a rule, taking place in order from the most caudal to the most cephalic ethmoidal concha (figs. 13, 14, 15, and 21).

As may be inferred from the above, the ethmoidal region on each side presents but for a brief time a single fold (fig. 13). As the nasal fossa enlarges superiorly and dorsally there is a differentiation in this region into two folds (fig. 14)—this change occurring approximately from the forty-eighth to the fiftieth day of fetal life. A later stage, 95 to 100 days, shows three well formed ethmoidal conchae (fig. 15). By the fourth month of fetal life the conchal field shows from three to four ethmoidal conchae, besides the concha inferior. Fetuses from the seventh month to term will, if carefully examined, show from *three* to *five* ethmoidal conchae (figs. 21 and 30). Of course in each case a corresponding number of furrows or meatuses are established—some of the meatuses, however, being very rudimentary. After birth the ethmoidal conchae and meatuses become reduced in number. This reduction is not carried so far in some cases as in others—thus accounting for the supernumerary or additional conchae and meatuses of many adult noses. Doubtless the number of ethmoidal conchae that are differentiated before birth have an important bearing on the number of conchae that may be present in an individual case—this is probably just as important a factor in determining the number of adult ethmoidal conchae as is the retrogressive change of reduction.

*The early changes in the meatus nasi medius*

Thus far only the superficial aspects of the lateral nasal wall, with especial reference to the development of folds and furrows (conchae and meatuses), have been considered. The general plan of these conchae and meatuses will be considered in detail in subsequent paragraphs. If now we return to a 40- to 50-day

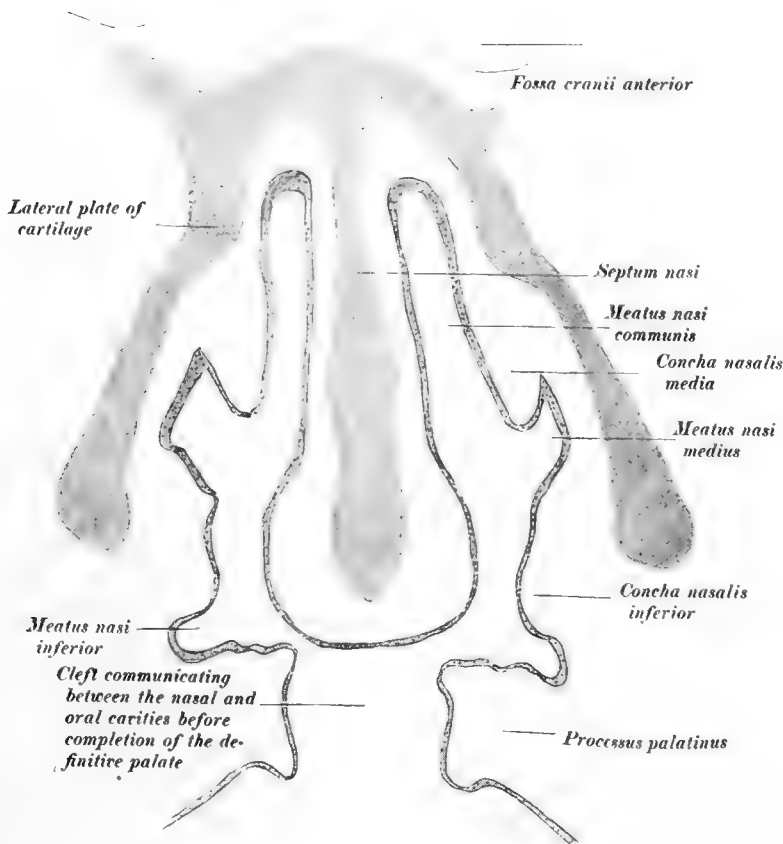


FIG. 16 ( $\times 26.6$ ) Outline drawing of a frontal section of the nasal cavity of an embryo aged approximately 49 days (Human embryo, No. 28, Cornell University series, slide 48). The nasal and oral cavities are in communication with each other at this point, *i.e.*, the palatal processes have not as yet coalesced in the median plane in the formation of the definitive palate. Note also the simple lateral wall of the middle nasal meatus—compare with fig. 18.

embryo and examine the portion of the middle meatus operculated by the middle nasal concha, we will notice that the lateral wall of this meatus is perfectly even (fig. 16). This study is best made by an examination of serial frontal sections through this region of the nose. This same region in an embryo aged from 58



to 60 days will indicate the beginning formation of the complicated nature of the meatus nasi medius—the most complex of all the meatuses. This change in the middle meatus is heralded by the appearance of a somewhat crescentic-shaped fold of mesenchyme and mucous membrane, set off by a slightly earlier grooving immediately superior to the fold. The latter has its free and rather sharp border directed superiorly and dorsally, and is the anlage of the processus uncinatus. The furrow immediately superior to the fold is the primitive infundibulum ethmoidale (fig. 18). It will be noticed that the furrow communicates rather freely with the meatus nasi medius through a rather widely open cleft—the hiatus semilunaris.

If now we examine a frontal section of a somewhat later embryo we will notice that the infundibulum ethmoidale early tends to develop beyond its primitive limits, and to pouch towards the ventral and superior portion of the meatus nasi medius, here ending blindly. This early, blind, ventral and superior extension of the infundibulum ethmoidale has been erroneously considered by some writers as the primitive sinus maxillaris (fig. 32). Of the bulla ethmoidalis there is nothing to be seen at this time.

About the seventieth day there is a slight, sometimes a comparatively extensive, pouching or evagination of the mucous membrane from the depth of the infundibulum ethmoidale—thus establishing the anlage of the sinus maxillaris. Shortly after this we have the first evidences of the bulla ethmoidalis appearing superior and lateral to the processus uncinatus—this in the form of very low accessory folds or conchae. By the beginning of the fourth month of fetal life the folds of the bulla are fairly well formed. At the end of the fourth month the folds with the bordering accessory furrows are in many cases well outlined (fig. 18). From some of the accessory furrows the so-called middle group of ethmoidal cells develop, and in a sense the former are the anlages of the latter structures. At a comparatively early stage we have evidences of a beginning extension of the middle meatus in a ventral and superior direction. This is the anlage of the recess, which Killian in later fetuses terms the 'Recessus frontalis' and it is in reality the first step in the formation of the sinus frontalis

and the most ventral of the anterior group of ethmoidal cells. The accessory folds and furrows, and the frontal recess of the middle meatus will be considered in detail in a subsequent portion of this paper.

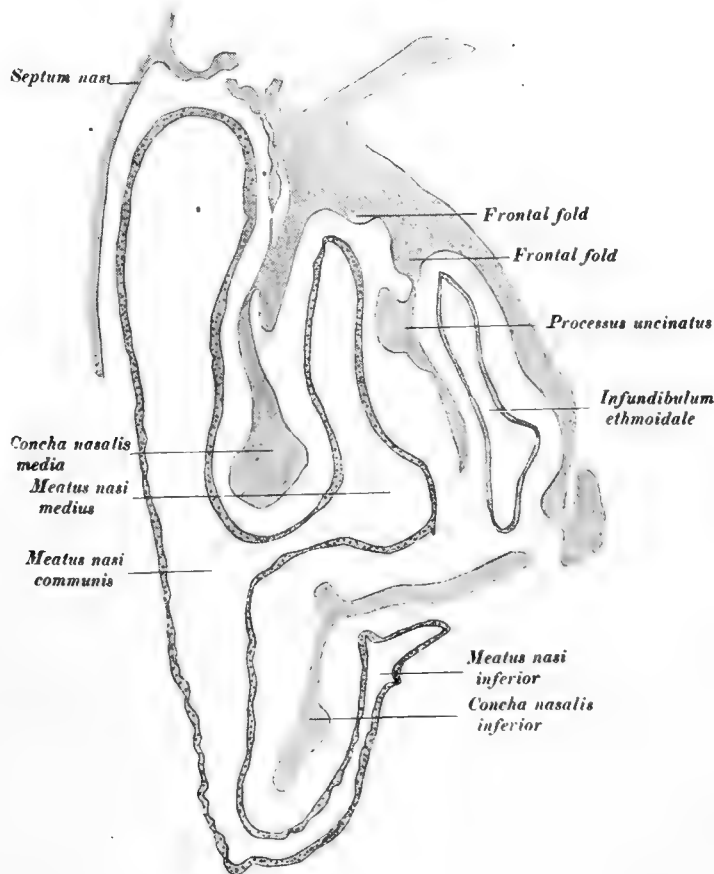


FIG. 17 ( $\times 10$ ) Outline drawing of a frontal section through the nose of an embryo aged 120 days (Human embryo, No. 42, Cornell University series, slide 29).

At this plane of section the processus uncinatus is fused with a frontal fold, and the infundibulum ethmoidale ends blindly. Compare this figure with fig. 18. In the latter figure the plane of section is farther dorsal.

#### *The early changes in the meatus nasi superior*

With the appearance of the accessory folds (conchae) and the frontal recess of the middle meatus, we have the establishment, in very many cases, of the anlage of an accessory fold (concha) on the lateral wall of the superior meatus. This fold early tends

to form very shallow recesses superior and inferior to it, and occasionally it has been mistaken for the superior nasal concha (fig. 19). From the superior and inferior recesses, and from the ventral and superior extremity of the superior meatus, most of the posterior group of ethmoidal cells develop—a matter which will be taken up subsequently. Frequently a posterior ethmoid cell develops from the meatus nasi suprema I. The latter meatus, however, is present in only 62.5 per cent of my adult specimens

*The early changes in the superior and dorsal portion of  
the nasal fossae*

A frontal section through the dorsal portion of the nasal cavity in a three-months embryo will already indicate the manner of constriction of a portion of the dorsal and superior part of the nasal fossae in the formation of the *early* anlagen of the sinus sphenoidales. In a 120-day embryo the sphenoidal-sinus anlagen are well advanced, but have not reached, or at least not extended into, the body of the developing sphenoid bone. The lateral nasal plates of cartilage more or less overhang the sphenoidal-sinus anlagen (fig. 20).

*The number of ethmoidal conchae and meatuses that are  
differentiated before birth*

As to the number of ethmoidal conchae and meatuses that are differentiated during the latter months of intrauterine life conflicting opinions are held. The meatus inferior and the concha inferior are constant, hence they will not be considered in the present connection.

Zuckerkindl states that three and at the highest four ethmoidal meatuses are formed (including the meatus medius). This would indicate three and at the highest four ethmoidal conchae. In reference to the latter he says: "Drei Siebbeinmuscheln repräsentieren . . . die typische Faltungsweise des Siebbeines." Killian, whose researches on this point are extensive, states that in fetuses of the ninth to the tenth month, five and even six eth-

moidal meatuses (Hauptfurchen) are present. He also finds a corresponding number of ethmoidal conchae (Hauptmuscheln), but includes among the number, as his "erste Hauptmuschel," the agger nasi (nasoturbinal) plus the processus uncinatus. Kilian comes to the following conclusion: "Mit dem, was das auf-

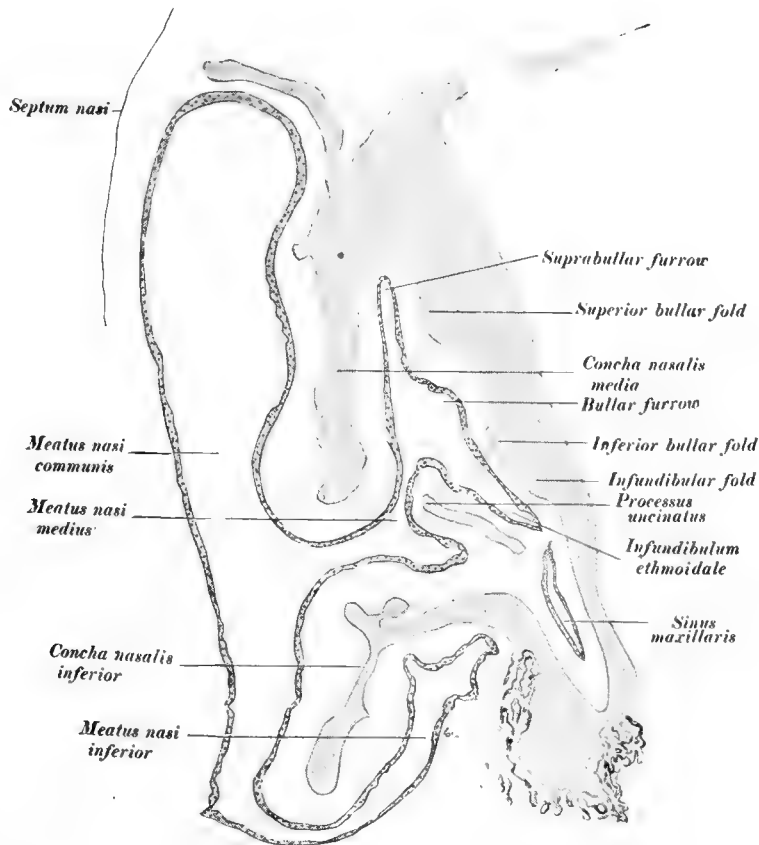


FIG. 18 ( $\times 10$ ) Outline drawing of a frontal section through the nose of the same embryo as that in fig. 17. The plane of section in this figure (slide 40) is farther dorsal, and is in the region of the bullar folds.

The processus uncinatus projects free at this plane of section, and the infundibulum ethmoidale communicates rather freely with the meatus nasi medius. The cartilage of the processus uncinatus is not continuous with the cartilage of the lateral plate at this level—compare this with the cartilage of the processus uncinatus in fig. 17.

merksame Studium meiner Präparate ergibt, stimmen die Beobachtungen an meiner ganzen Material überein, wobei ich bemerke, dass die erste, zweite und dritte Hauptfurchen fast regelmässig vorkommen und dass die vierte in über 30 Fällen, die fünfte und sechste in noch einigen weiteren Beispielen vertre-

ten sind." Kallius holds that seldom are all six furrows differentiated. Mihalkovics says that "Knorpelige Muscheln sind beim Menschen im embryonalen Leben stets 4 vorhanden, oft auch 5." The latter, however, includes among this number the concha nasalis inferior, which has not been done by the other writers mentioned, since they considered the ethmoidal region only.

After an extended study of the ethmoidal region in fetuses, I find that there is more or less uniformity up to the fourth month. However during the latter half of fetal life there is indeed great variation in the number and form of the ethmoidal conchae and meatuses. Notwithstanding this I think that careful analyses indicate a general plan more or less common to most ethmoidal regions—some specimens differing here and others there from the plan. Occasionally some specimens differ so markedly from this general plan that they are difficult of comparison.

Doubtless the personal equation is quite a factor in determining the number of ethmoidal conchae. In many cases some of the conchae are so rudimentary that one observer might not consider the under-developed folds as individual conchae, yet another observer would include them among the number.

I find by careful study of the ethmoidal region in a series of specimens from late fetuses that five ethmoidal conchae are not uncommon, however the most superior and dorsal conchae may be but faintly outlined. We occasionally find all five conchae well marked (fig. 21). In this respect my observations agree with those of Killian—he giving six as his highest number of ethmoidal conchae. The apparent discrepancy in number is due to the fact that he (Killian) names the agger nasi (nasoturbinal) plus the processus uncinatus as his 'erste Hauptmuschel.' On the other hand I do not do this, but consider his 'zweite Hauptmuschel' as my concha nasalis media. I find four ethmoidal conchae very frequent indeed, in fact fully 65 per cent of later fetuses, according to my series of specimens, present four (fig. 26). It must, however, be remembered that some of the conchae or folds are extremely rudimentary, nevertheless they must be taken into consideration when analyzing the ethmoidal region. Occa-

sionally some specimens show a low degree of differentiation and possess but three ethmoidal conchae (fig. 28).

It is indeed difficult to say whether in the latter cases the progressive changes (differentiation) have not been carried so far as in some others, or whether retrogressive changes (coalescence of two or more conchae with obliteration of the intervening furrows)

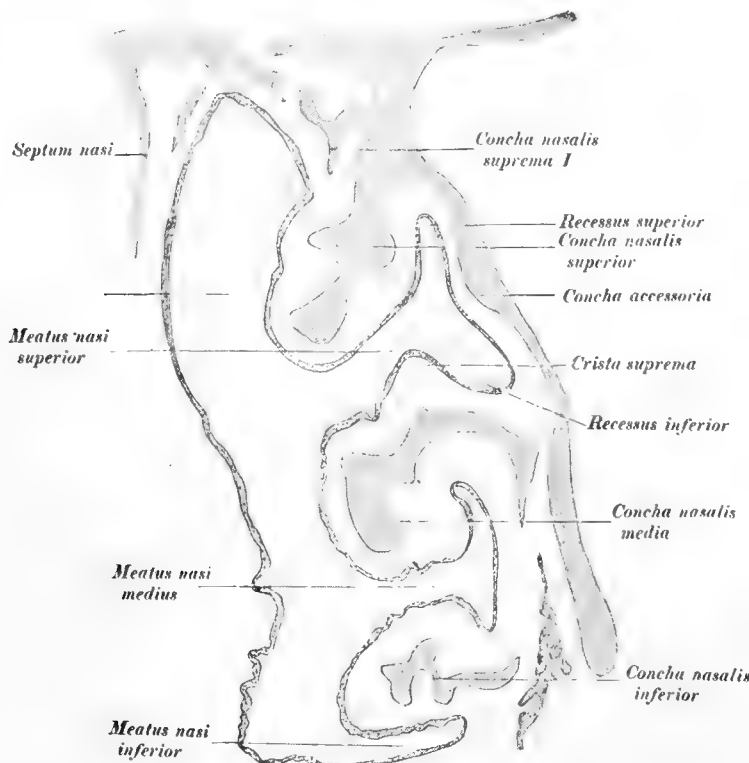


FIG. 19 ( $\times 10$ ) Outline drawing of a frontal section through the nose of the same embryo as those in figs. 17 and 18. This section (slide 67) is in the region of the accessory concha of the meatus nasi superior, hence is farther dorsal than that in fig. 17.

Shows how the crista suprema of the concha media aids in forming the recessus inferior of the meatus superior. Compare the meatus superior of this section with the meatus medius of section, fig. 18.

took place at an earlier time. The latter is doubtless an active process after birth, because we rarely find more than three ethmoidal conchae in the adult. It is, therefore, quite possible that retrogressive changes are instituted much earlier in some cases, and that the ethmoidal regions which have a comparatively small number of conchae in the late fetus, originally possessed a larger number of folds. I, however, believe that in the majority of these

cases the differentiation in the ethmoidal region is not carried so far as in some others, hence a smaller number of ethmoidal conchae.

Whatever the number of ethmoidal conchae may be, there is a corresponding number of meatuses or furrows; *i.e.*, each concha more or less overhangs a furrow or meatus. This overhanging is especially marked in the meatus nasi medius. Killian gives six

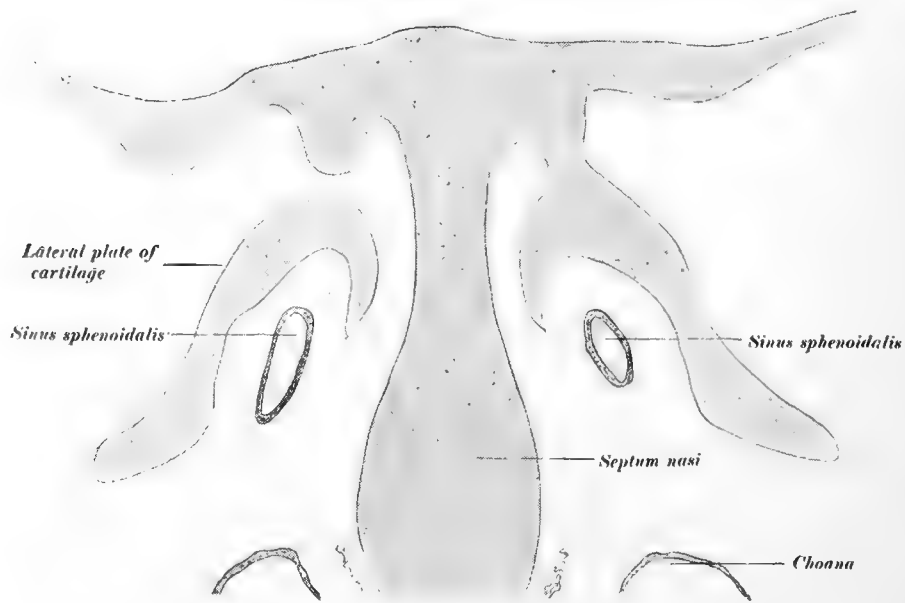


FIG. 20 ( $\times 10$ ) Outline drawing of a frontal section through the dorsal portion of the nasal cavity. The section (slide 91) is from the same embryo as are those represented in figs. 17, 18, 19. Note how the dorsal and superior portions of the nasal fossae establish the anlagen of the sinus sphenoidales.

as his highest number of 'Hauptfurchen'—this would place a meatus dorsal and superior to the most cephalic concha present. I, however, prefer to think of this region as belonging to the recessus sphenothmoidalis. The latter because it corresponds to the position of the adult recessus sphenothmoidalis; also that after birth we do not usually think of the meatus nasi as being superior to, but inferior to the corresponding conchae nasales. The recessus sphenothmoidalis, however, is very closely related to the meatus nasi suprema I, and in the absence of the latter with the meatus nasi superior.

*Ethmoidal nomenclature*

Owing to the *comparatively* large number of ethmoidal conchae and meatuses present in the nose of the late fetus in comparison to adult conditions, it will be necessary for the sake of description to adopt some nomenclature sufficiently extensive to cover the extreme cases one meets at this time.

Zuckerkindl's nomenclature is not extensive enough to cover the fetal field in all cases. He designates the ethmoidal conchae as "untere, mittlere, obere, und vierte (oberste) Siebbeinmuscheln." He speaks of the ethmoidal meatuses as "inferior, superior, und suprema Fissurae ethmoidales." He, however, complicates his nomenclature by his views on the "vierte (oberste) Siebbeinmuschel." He makes the following statements:

"In 6.7 Percent der Fälle tritt bei Kindern und Embryonen noch eine vierte Siebbeinmuschel auf, die sich zwischen die mittlere und obere Concha ethmoidalis einschibt. . . . Vierte Siebbeinmuschel (Concha suprema), begrenzt: unten von der Fissura ethmoidalis suprema, oben von der Siebplatte."

There is doubtless a discrepancy in the above statements. If the "vierte (oberste) Siebbeinmuschel" inserts itself ("einschiebt") between the "mittlere und obere Concha ethmoidalis," then the furrows or meatuses that border it are his (Zuckerkindl's) "superior und suprema Fissurae ethmoidales," and the "obere Siebbeinmuschel" would be between his "Fissura ethmoidalis suprema" and the "Siebplatte." The reader will at once notice the difficulty encountered in attempting to compare this with other nomenclatures.

Killian adopts a nomenclature to cover, according to my specimens, all cases, by naming the ethmoidal meatuses, including the meatus medius, 'Hauptfurchen<sub>1-6</sub>;' and the ethmoidal conchae, 'Hauptmuscheln<sub>1-6</sub>.' He, however, includes among his "Hauptmuscheln" the agger nasi (nasoturbinal) plus the processus uncinatus as his 'erste Hauptmuschel,' which has not been done in this paper.

Peter also covers the field by designating the ethmoidal conchae 'Ethmoturbinalia<sub>1-5</sub>.' The latter does not include Killian's 'erste Hauptmuschel' among this number.



Schönemann speaks of the concha media as the 'Basoturbinale,' and Paulli in an exhaustive study refers to all the major nasal conchae as 'Endoturbinalien,' in contradistinction to the accessory conchae which he designates as 'Ektoturbinalien.'

The general text-books of anatomy, also the BNA (nomina anatomica, Basel), offer nomenclatures sufficient for practically all adult conditions, but not for the late fetal stage. In order not to depart too far from the general texts on anatomy and the BNA, yet offer for late fetal conditions a sufficiently comprehensive nomenclature, I will in subsequent paragraphs use the following names for the several nasal conchae and meatuses.

The nomenclature offered is not necessary for all cases, nevertheless it is essential to have a terminology that covers not only the adult, but also the extreme fetal conditions one frequently meets.

Nomenclature used in this paper:

<i>Meatus nasi.</i>	<i>Conchae nasales.</i>
Meatus nasi inferior,	Concha nasalis inferior,
Meatus nasi medius,	Concha nasalis media,
Meatus nasi superior,	Concha nasalis superior,
Meatus nasi suprema I,	Concha nasalis suprema I,
Meatus nasi suprema II,	Concha nasalis suprema II,
Meatus nasi suprema III.	Concha nasalis suprema III.

*The nasal meatuses and conchae as seen during the latter stages of intrauterine life*

The nasal meatuses and conchae of the fetus of the latter half of intrauterine life may now be considered in detail. The ethmoidal region is rather complex in construction, and with the accessory folds (conchae) and furrows, the anlagen of the paranasal chambers, and the conchae and meatuses, offers an interesting field for study from the second month on to term. The meatuses will be taken up first for detailed study, followed by the nasal conchae. After this the accessory folds (conchae) and the accessory furrows of the meatuses will be considered; finally the anlagen of the paranasal chambers.

*The nasal meatuses*

*The meatus nasi inferior.* The meatus nasi inferior is one of the first meatuses to become well established. It is primitively formed between the anlage of the concha inferior and the primitive processus palatinus. Later, with the fusion of the two palatal processes in the median plane to form the definitive palate, the meatus inferior comes to occupy the position between the concha inferior and the floor of the nasal fossa. The ductus nasolacrimalis connects with the meatus inferior approximately at term. A reference to fig. 37 will, however, show a frontal section of the left nasal fossa of a term fetus in which the barrier between the meatus inferior and the ductus nasolacrimalis is still intact, *i.e.*, the duct has not acquired a lumen in the region of the meatus inferior. Finally there are two layers of abutting epithelium, one nasal and the other that of the duct, forming the barrier. The membrane now seems to thin out and to become attenuated, ultimately rupturing; in other words the duct becomes patent at this point. This attenuation of two layers of abutting epithelium, ultimately resulting in rupture, in order that communication between two cavities may be established, reminds one somewhat of the attenuation and rupture of the membrana buconasalis.

The meatus nasi inferior is otherwise simple and offers no conditions that warrant a further consideration at this time.

*The meatus nasi medius, superior, suprema I, suprema II, suprema III.* The ethmoidal meatuses, including the meatus medius—since they have many things in common—may for the time be considered together. It must be recalled that in many cases the meatus suprema I, II, and III are extremely rudimentary, and are merely very shallow, short grooves. Again in many instances the meatus nasi suprema II and III are not at all differentiated. The meatus nasi suprema I is the most constant of the supreme meatuses. While we must follow some general type for the sake of description, it does not necessarily indicate that all specimens agree in their entirety.

The ethmoidal meatuses all converge at their inferior and dorsal extremities in the region of the angle formed by the junction

of the anterior and inferior surfaces of the body of the sphenoid bone (fig. 21). These meatuses in a general way possess knees or bends, thus presenting ascending and descending rami. These bends are best marked in the middle and superior meatuses. It is a constant condition in the middle meatus, and a fairly large number of specimens show a well marked ascending ramus for the superior meatus. The remaining meatuses when present, however, do not have well marked ascending rami. In fact very many specimens give no evidence whatever of individual ascending rami for the meatus suprema I, II, and III (fig. 23). In a general way it may be stated that the differentiation of ascending rami becomes gradually less marked as we pass from the meatus medius to the meatus suprema III (figs. 21, 23, and 25).

*The ascending rami.* The ascending ramus of the middle meatus is directed rather obliquely, in a ventral and superior direction—this is less so for the corresponding ramus of the superior meatus. As we pass from the superior meatus to the supreme meatus III we find a rapid change in direction of the ascending rami to a more or less vertical plane, *i.e.*, the ascending rami more nearly vertical to the cribriform plate of the ethmoid bone. We may, therefore, say—when ascending rami are differentiated—that as we proceed from the meatus medius to the meatus suprema III, there is a gradual change in direction of the ascending rami from an oblique to a more or less vertical plane (fig. 21).

In fig. 21 we have the representation of a specimen which presents an ascending ramus for each corresponding meatus. The ascending rami of the supreme meatuses, II and III are, however, not marked, in that the two latter meatuses seldom have well developed knees. In fig. 22 the ascending rami are indicated merely by very shallow, short grooves near the cribriform plate of the ethmoid bone. In the latter case in no instance does an ascending ramus reach the corresponding descending ramus. This doubtless means a lessened degree of differentiation into ethmoidal conchae in the region of the ascending rami as compared with the corresponding region in fig. 21.

Sometimes there is apparently no attempt at the formation of individual conchae in the region of the ascending rami, hence in these cases the meatuses—with the exception of the meatus medius—do not possess individual ascending rami (fig. 23). In other instances the meatus superior and medius have ascending rami, but the remaining ethmoidal meatuses do not (fig. 25). The ascending ramus of the meatus superior is occasionally so well developed that it reaches nearly to the cribriform plate of the ethmoid bone (fig. 24).

A reference to figs. 21 to 30 will indicate the irregularity and inconstancy of the ascending rami, with the single exception of the meatus medius. Notwithstanding this inconstancy of the ascending rami, we occasionally have ascending rami as well marked as the descending rami (fig. 24). Rarely we find them all fairly well marked (fig. 21), but in the vast majority of cases the ascending rami of the supreme meatuses vary from an extremely rudimentary state to a complete absence. The number of times the ascending rami are present in some form, however, justifies the division of the ethmoidal meatuses into ascending and descending portions.

*The descending rami.* The descending rami of the ethmoidal meatuses are as a rule much better differentiated than are the corresponding ascending rami. What has been said of the ascending rami, *i.e.*, a gradual change from an oblique to a more or less vertical plane, as one passes from the meatus medius to the meatus suprema III, applies also, although with less degree, to the descending rami.

The descending ramus of the meatus medius lies practically in a horizontal plane, and the corresponding ramus of the meatus superior varies from it but little in its general direction (fig. 22). On the other hand, the descending rami of the meatus suprema I, II, and III assume a more vertical direction—the latter being most marked in the meatus suprema III (fig. 21). In very many cases the descending rami of the meatus suprema II and III are extremely rudimentary or not at all differentiated (fig. 29). The descending ramus of the meatus suprema I is present in some form, according to my specimens, in practically

all cases. Of course after birth the meatus suprema I in many cases becomes obliterated (62.5 per cent of my adult specimens present a meatus suprema I) and in the adult the concha nasalis superior and suprema I are represented by the concha superior. The meatus medius and superior are, of course, constant in both the fetus and in the adult.

In a general way we may say that the integrity of the ethmoidal meatuses present, with the possible exceptions of the meatus medius and superior, depends practically wholly upon the differentiation and degree of development of the descending rami, and not upon the ascending rami (figs. 21 to 24).

### *The nasal conchae*

*The concha nasalis inferior.* The concha nasalis inferior appears first in the formation of nasal conchae, and for some time occupies the greater portion of the lateral nasal wall (fig. 13). It is at first not well differentiated, but with the growth of the fold and the deepening of the furrows (meatuses) inferior and superior to it, the concha becomes more sharply outlined. With the growth of the nasal fossa in the dorsal and superior direction, and the resulting development of the ethmoidal region, the concha inferior gradually comes to take on the usual adult form. During the latter stages of intrauterine life it offers nothing of note to justify a further consideration of it in this connection.

*The agger nasi.* The agger nasi is homologous with the nasoturbinal which is so well developed in some other forms (pig, cow, sheep, dog, skunk, etc.). It is extremely rudimentary in man in comparison, for example, to the corresponding structure in the pig, rabbit, cow, etc. The agger nasi is an elevation located ventral to the concha nasalis media, and ventral and superior to the concha nasalis inferior. It is more or less parallel to the bridge of the nose. It is at times fairly well developed in the late fetus, and again we find it extremely rudimentary (figs. 22, 25, 29, and 39).

Killian, in conjunction with the processus uncinatus of the ethmoid bone, considers it as an ethmoidal concha, *i.e.*, belonging to the ethmoidal group of conchae. He considers the processus

uncinatus as the descending crus of the agger nasi, and speaks of the two structures as his "erste Hauptmuschel." In commenting on Killian's classification of the nasoturbinal (agger nasi), Peter, who studied the structure from a comparative point of view, says: "Killian und vor ihm Andere glaubten auch dass das Nasoturbinale in das Schema der Riechmuscheln einzwängen zu müssen und suchten daher nach einem Crus descendens desselben, den sie im Processus uncinatus gefunden zu haben meinten. Nach obiger Darstellung der Muschelentwicklung ist diese Forderung ebenso unberechtigt, wie sie für das Maxilloturbinale wäre."

I prefer to think of the processus uncinatus as an accessory concha, and to class it with the folds of the bulla ethmoidalis (also accessory conchae of the middle meatus) and with the accessory concha of the superior meatus. While the processus uncinatus is more or less continuous with the agger nasi, and for descriptive purposes might be considered as the descending crus of the agger nasi, it is also in many instances fused ventrally and superiorly with the lateral surface of the concha nasalis media. I have also seen it fused across the infundibulum ethmoidale with the ventral extremity of the bulla ethmoidalis. In subsequent paragraphs I will consider the concha nasalis media as Killian's "zweite Hauptmuschel," and will not consider the agger nasi and the processus uncinatus as belonging to the regular group of ethmoidal conchae.

*The concha nasalis media, superior, suprema I, suprema II, suprema III.* The regular group of ethmoidal conchae have many things in common, hence may for the time be considered together. In a general way they all possess knees or bends, thus presenting superior and inferior, or better, ascending and descending portions (Crura ascendens and descendens of Killian). As we pass from the concha media to the concha suprema III, we find a gradual lessening in the degree of the knee or bend. The supreme conchae represent practically straight angles, hence no well marked division into ascending and descending crura, *i.e.*, the conchae are practically straight. This is also aided in very many cases by the rudimentary development or complete absence of individual ascending crura (figs. 23 and 29). As we pass from the concha

## EXPLANATION OF FIGURES

FIGS. 21, 22, 23 ( $\times 1.2$ ) Drawings of specimens of the lateral nasal wall from the anatomical series, Cornell University. The drawings all represent the lateral nasal wall of term fetuses.

Compare especially the regions of the ascending crura of the ethmoidal conchae in the three figures. In fig. 21 the ascending and the descending rami of the ethmoidal meatuses are continuous. In fig. 22, however, the very rudimentary ascending rami of the ethmoidal meatuses are not continuous with the descending rami—save in the meatus medius. It will be noticed in fig. 23 that there is no attempt at the differentiation of individual ascending crura for the ethmoidal conchae; the concha media is, however, an exception. In the latter figure the ascending crura are represented by a general ascending-crural mass undifferentiated.

Note the extensive furrows on the medial surface of the concha nasalis media in figs. 21 and 22.

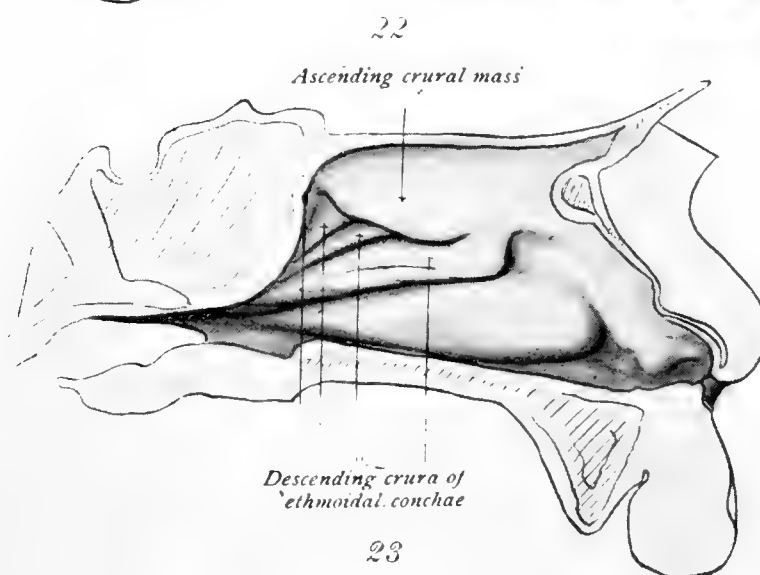
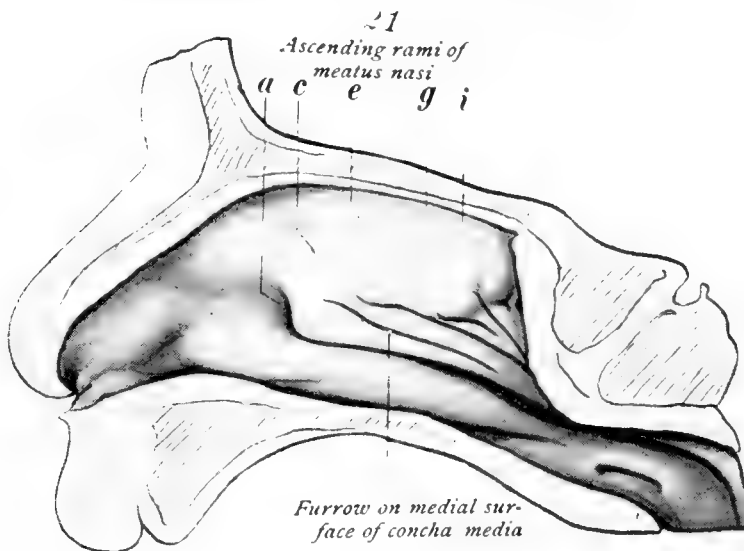
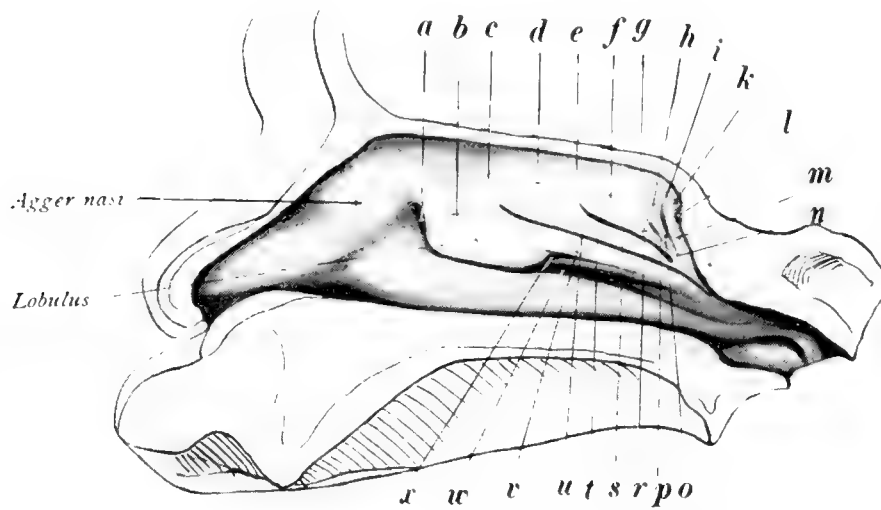
*a, c, e, g, i* = ascending rami of the ethmoidal meatuses; *w, u, s, p, n* = descending rami of the ethmoidal meatuses; *b, d, f, h, k* = ascending crura of the ethmoidal conchae; *v, t, r, o, m* = descending crura of the ethmoidal conchae; *l* = sinus sphenoidalis; *x* = Incisura retrolobularis.

FIGS. 24, 25, 26 Drawings of specimens of the lateral wall of the nasal cavity from the anatomical series, Cornell University.

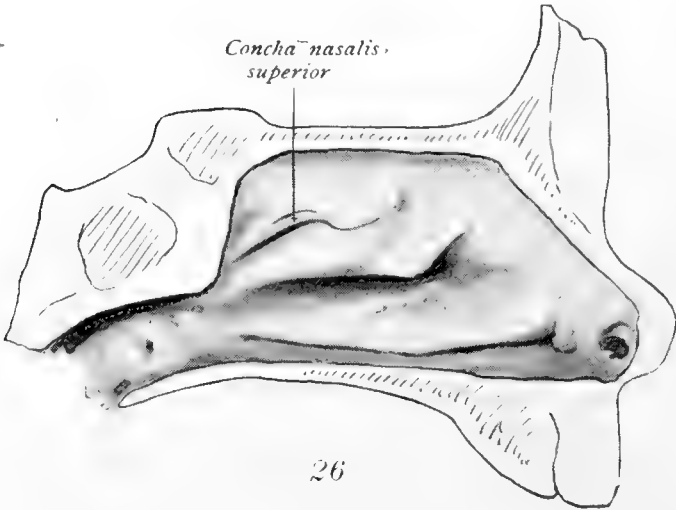
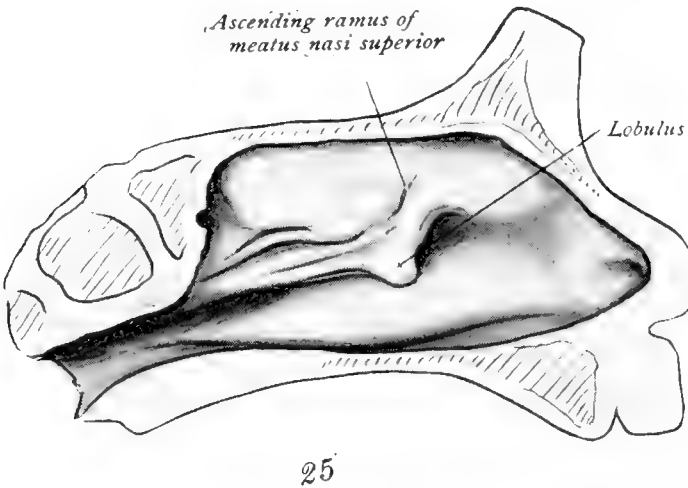
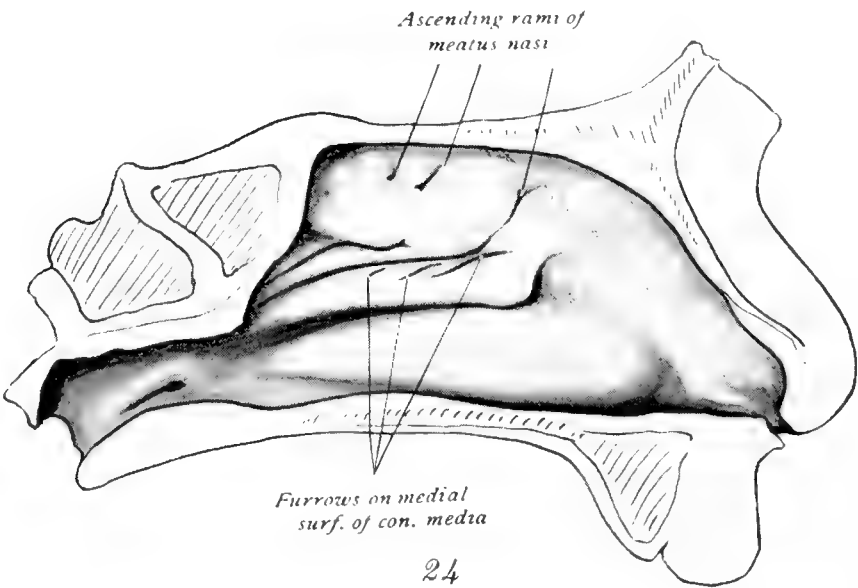
FIG. 24 ( $\times 1.2$ ) From a fetus at term. Shows a marked ascending ramus for the meatus nasi medius. The other ascending rami present are less developed and do not reach the corresponding descending rami. Note also the multiple furrows on the medial surface of the concha nasalis media.

FIG. 25 ( $\times 1.2$ ) From a 7-months fetus. To show the furrows on the concha nasalis media and the lobule in the region of the knee. There is little or no differentiation in the region of the ascending crura for the concha superior and the concha suprema I.

FIG. 26 ( $\times 1.8$ ) From a 190-day fetus. The differentiation into individual ethmoidal conchae has not progressed very far at this early period. Compare with fig. 21.







media towards the concha suprema III we find a gradual change in the plane of the conchae, from a more or less oblique direction to a more or less vertical one (figs. 21 and 23).

*The ascending crura.* The ascending crura always tend more towards the perpendicular than do the corresponding descending crura. This is especially true of the concha media and superior,

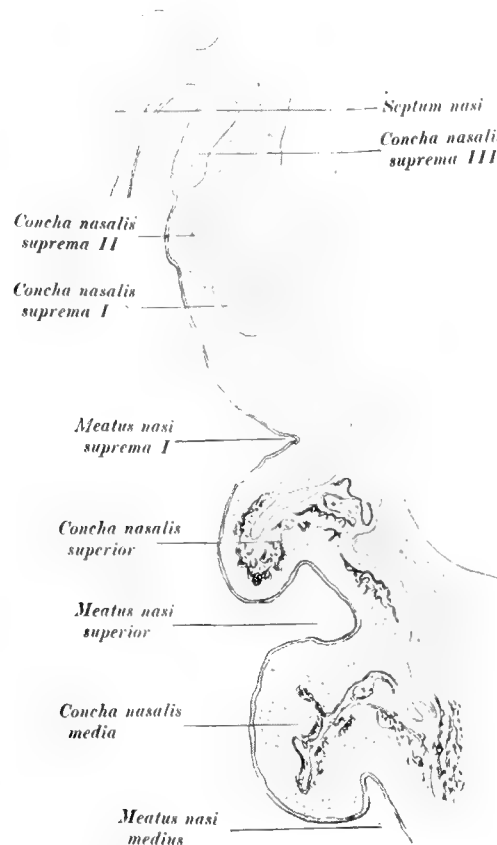
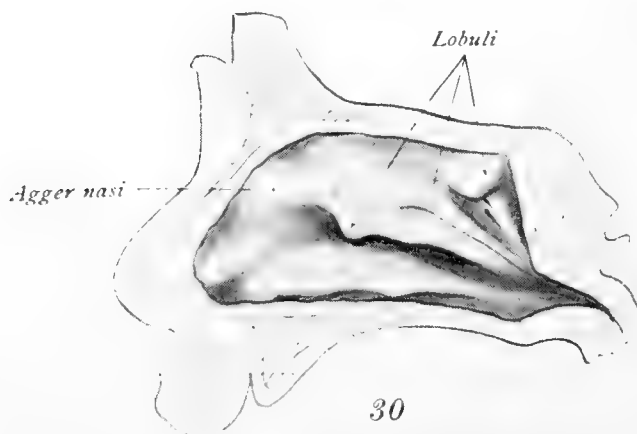
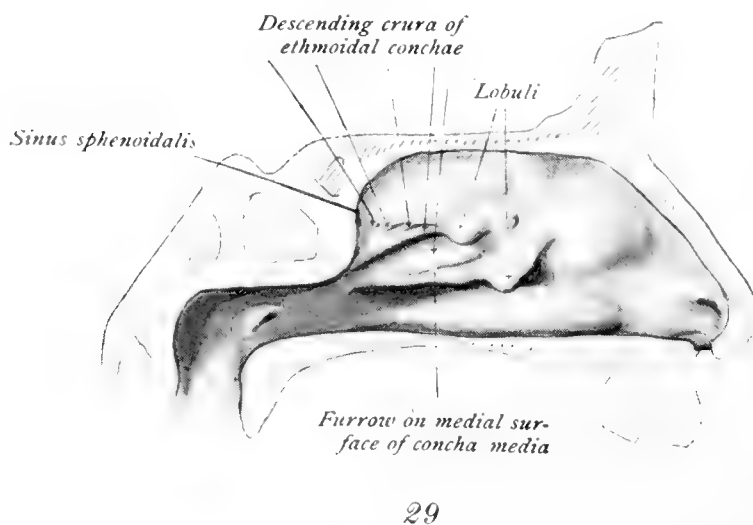
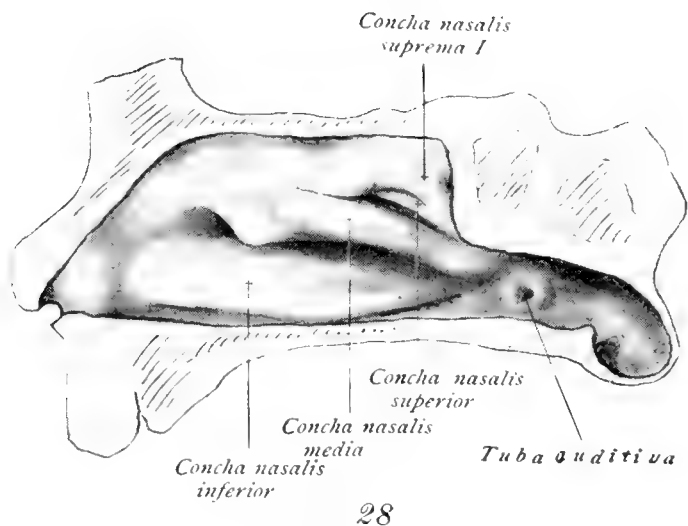


FIG. 27 ( $\times 8$ ) Drawing of a frontal section through the lateral wall of the nasal cavity in the region of the supreme conchae (fetus aged from 7-8 months, series B, slide 48). The concha nasalis inferior is not included in the section.

also at the times of the concha suprema I (figs. 21 and 24). Fig. 21 shows five fairly well marked ascending crura. It will be noticed that they are almost perpendicular to the cribriform plate of the ethmoid bone. However their superior extremities as individual conchae do not closely approach this plate. We may either think of the conchae as fused into one mass, or that differentiation into individual conchae stopped short of the cribriform plate. The



FIGS. 28, 29, 30 Drawings of specimens of the lateral wall of the nasal cavity from the anatomical series, Cornell University.

FIG. 28 ( $\times 1.8$ ) From a 190-day fetus. Note especially the rudimentary character of the concha superior. The accessory concha of the superior meatus cannot be seen.

FIG. 29 ( $\times 1.2$ ) From a 235-day fetus. Note the marked differentiation into descending crura of the ethmoidal conchae. The ascending crural mass is as yet undifferentiated.

FIG. 30 ( $\times 1.2$ ) From a 210-day fetus. Note especially the lobuli on the ethmoidal conchae.

tendency of the conchae to become straighter as one proceeds from the concha media to the concha suprema III is well illustrated in this figure (21).

In other cases the individuality of the ascending crura is only faintly indicated by very shallow grooves throwing into slight relief extremely rudimentary crura. In fig. 22 we have such an example. Here we find the general ascending-crural mass well developed, but the differentiation into five ascending crura, with the single exception of the ascending crus of the concha media, only slightly marked. In some instances the ascending crus of the concha media stands out in bold relief. This is especially due to a well developed ascending ramus of the meatus superior (fig. 24). In the latter figure the ascending ramus of the meatus superior closely approaches the cribriform plate of the ethmoid bone.

Occasionally the whole ascending-crural mass presents an even and unbroken surface, there being no furrows or grooves to throw any portion of the mass into relief. In such instances there is no differentiation of the mass into individual ascending crura; however we may consider this mass as representing the ascending crura undifferentiated (fig. 23). If not the latter, then we must say that the individual ascending crura coalesced into a general fold or mass at an earlier stage. The former theory is, however, the most plausible according to my observations. The general ascending-crural mass is often more or less overhanging in character. Again we may have the ascending crura of the concha media and superior well outlined, while the supreme group (J, II, III) is represented by one fold (fig. 29).

A reference to figs. 21 to 30 will show some of the different combinations met with in the fetal specimens examined for the substance of this paper.

*The descending crura.* The descending crura of the ethmoidal conchae are, almost without exception, better differentiated than are the corresponding ascending crura. The former also occupy a more nearly horizontal plane; this, however, lessens as we approach the concha suprema III. The middle and superior conchae present the best marked descending crura. The concha suprema I also frequently possesses a well marked descend-

ing crus. The descending crura of the conchae supremae II and III, are variable and frequently entirely wanting. At times the latter two are fused into one, and then in turn closely associated with the anterior surface of the body of the sphenoid bone, in the region of the developing sinus sphenoidalis. The concha suprema III loses its identity early by becoming associated with the anterior surface of the body of the sphenoid bone.

*Lobules and nodules of the ethmoidal conchae.* At the junction of the ascending and descending crura, or in the region of the knees, we frequently find overhanging lobule formations. This is a fairly constant condition for the concha media, and it is also very common for the concha superior (figs. 21 and 28). Occasionally the concha suprema I also presents a similar formation, which in some cases more or less overhangs the supreme meatuses and conchae. In the latter case it really amounts to the ascending crura of the supreme conchae (fig. 30). Schwalbe in his "Anatomie der Sinnesorgane," refers to the lobule of the concha nasalis media as the 'Operculum meatus narium medii.' He does not, however, refer to similar formations of some of the other conchae. Frequently there is a secondary thickening on the middle of the lobules. Zuckerkandl calls attention to this for the lobule of the concha media; however the other lobules frequently present the same condition (figs. 25 and 30). Killian refers to this secondary prominence as the 'Nodulus lobuli.' Fig. 30 shows three well formed lobules—the superior one more or less operculating the conchae and the meatuses in the immediate vicinity. The lobule of the concha media is at times very prominent, and it takes on different shapes as is evidenced by figs. 21 and 25.

My studies have led me to agree with the statement of Peter, namely, that "Dieser Lobulus mit Nodulus ist der vorderen Spitze der Ethmoturbinalia der Säugetiere zu vergleichen."

*Grooves on the concha nasalis media.* The concha nasalis media needs further consideration separately, since it presents grooves or furrows on its medial and inferior surfaces (figs. 22, 23, and 25). These furrows which show on the mucous membrane covering the concha, generally present corresponding depressions in

the conchal cartilage or bone. At times we find marked furrows on the conchal cartilage yet the surface remains unfurrowed in these positions. Killian concludes, therefore, that "der Knorpel scheint demnach weit conservativer in der Bewahrung der ursprünglichen Form zu sein, als die Schleimhaut."

The two most constant of these furrows according to my specimens are what may be termed, (1) the superior and (2) the inferior (fig. 25). In fig. 22 we have an extensively developed superior furrow. It more or less splits the descending crus of the concha media into superior and inferior portions. The latter condition led some former investigators (Zuckerkandl and others) erroneously to consider the deep furrow as one of the chief ethmoidal furrows, and the portion of the concha media superior to the furrow, as a separate ethmoidal concha (mittlere Siebbeinmuschel, Zuckerkandl). Seydel later showed the error of this contention, and Zuckerkandl subsequently retracts from his former view in the following words: "Dagegen möchte ich das Gleiche für die untere Siebbeinmuschel nicht mehr aufrecht erhalten, denn wir haben erfahren, dass die mittlere Siebbeinmuschel nicht nur aus einer Teilung der Concha ethmoidalis inferior hervorgeht. . . . und stimme O. Seydel bei, der die untere Siebbeinmuschel als Repräsentantin eines einzigen Riechwulstes ansieht."

Killian refers to the furrows of the concha media as 'Nebenfurchen,' and the notch formed by the inferior furrow differentiating to the free inferior border of the concha media, dorsal to the lobule of the knee, as the 'Incisura retrolobularis' (fig. 21). Sometimes the superior furrow is broken into three or more grooves, rather obliquely placed, the ventral one being as a rule the best marked (fig. 24).

#### *Accessory nasal folds (conchae) and furrows*

Upon turning superiorly the concha nasalis media of the adult nose we expose for study the bulla ethmoidalis and the processus uncinatus—accessory conchae of the meatus nasi medius. By removing the concha nasalis superior we also expose in very

many cases an accessory fold or concha on the lateral wall of the meatus nasi superior which is comparable to the bulla ethmoidalis. These structures contained in the middle and the superior meatuses are hidden or accessory nasal conchae, comparable to the accessory conchae found in mammals. In the middle meatus we also note the hiatus semilunaris, and if the processus uncinatus be turned inferiorly, the infundibulum ethmoidale is exposed. In the superior and ventral portion of the middle meatus we note varying relations in different specimens, between the nasofrontal duct and the infundibulum ethmoidale and some of the anterior ethmoidal cells. The ostia of ethmoid cells are also to be seen in the meatus medius, superior, and suprema I—the relations of these ostia varying much in different specimens.

In order to interpret these varying adult conditions and relations, it is essential that we study the developmental changes in the middle and the superior meatuses at some length in the embryo, late fetus, and in the young child. This will at once point out the probabilities of development and the varying adult conditions one may expect to meet in a series of specimens.

*The accessory folds and furrows of the descending ramus of the meatus nasi medius.* If we examine serial frontal sections of the nose of a 40-day embryo we will find that the lateral wall of the meatus nasi medius is more or less even and simple. If, on the other hand, we examine the same region in embryos aged from 50 to 60 days we will find a somewhat crescentic shaped fold with its free border directed superiorly, breaking the evenness of the wall. The latter is the anlage of the processus uncinatus and the first of the series of accessory folds to appear on the lateral wall of the meatus medius. This fold at once aids in forming a furrow immediately superior to it—the primitive infundibulum ethmoidale. It is quite probable that the furrow first establishes an anlage and this in turn throws into slight relief a portion of the mucous membrane inferior to it, thus establishing the anlage of the processus uncinatus. It may, however, be said that both structures are more or less dependent upon each other in establishing anlages. The same principles are obviously here involved

just as they are in forming the primitive nasal meatuses and conchae.

From this furrow (primitive infundibulum ethmoidale) the sinus maxillaris develops its anlage in the form of an evagination of the mucous membrane. This maxillary sinus anlage begins to be established from the sixty-fifth to the seventieth day of embryonal life. In a former paper I suggested that primitively the pouching of the sinus maxillaris aided in deepening the infundibulum ethmoidale; thus causing the processus uncinatus to stand out better at an early period.

Shortly after this we have the first evidence of the bulla ethmoidalis, appearing superior and lateral to the processus uncinatus. The bulla ethmoidalis is first indicated by special thickenings (one or two) of the lateral plate of cartilage—the cartilaginous thickenings appearing on its medial surface. At first the bullar anlages do not cause the mucous membrane to bulge towards the lumen of the nasal cavity; hence these early stages pass unobserved unless one examine serial frontal sections through this region. Later, however, say in a 120-day embryo, the cartilaginous prominences have developed sufficiently to push the mucous membrane on the lateral wall of the meatus medius into relief (figs. 17 and 18). We now have established a fold, or folds, (bullar folds) lateral and superior to the processus uncinatus. If two folds appear there is an intervening furrow. These folds represent the primitive bulla ethmoidalis. Usually the intervening furrow, when present, disappears after birth. Occasionally, however, an ethmoidal cell develops from this furrow.

Thus far no mention has been made of another fold that appears in many cases inferior to the bullar folds and lateral to the infundibulum ethmoidale. Because of its relations to the infundibulum ethmoidale I shall speak of this as the infundibular fold. It is never very prominent and forms in part the lateral wall of the infundibulum ethmoidale. It may persist as a fold after birth, but it generally becomes leveled down to an even surface which imperceptibly passes on to the bullar surface.

It will, therefore, be seen that we have, during the latter half of intrauterine life, fairly well outlined grooves and folds on the



lateral wall of the descending ramus of the meatus medius.' If we take into consideration the extreme cases found in the late fetus we have thus formed four folds and four furrows. These folds may appropriately be named superior and inferior bullar folds, infundibular fold, and processus uncinatus; the furrows, the suprabullar furrow or recess, the bullar furrow, the infrabullar furrow, and the infundibulum ethmoidale. The suprabullar recess and the infundibulum ethmoidale are constant and are the most important furrows. The others are of less importance and are more irregular and inconstant in their development (figs. 31 and 34).

Killian, who has studied this region extensively, finds a similar number of folds and furrows on the lateral wall of the middle meatus. He, however, considers the processus uncinatus as the descending crus of his 'erste Hauptmuschel,' hence includes it in the class of regular ethmoidal conchae. My superior bullar, inferior bullar, and infundibular folds or conchae correspond to his 'obere, mittlere, und untere Nebenmuscheln,' respectively. My suprabullar, bullar, and infrabullar furrows, and infundibulum ethmoidale correspond to his 'Recessus superior, obere Zwischenfurche, untere Zwischenfurche, und Recessus inferior,' respectively.

*The suprabullar furrow or recess.* The suprabullar furrow or recess is practically constant. It varies somewhat in its form and extent, but all specimens give some evidence of it. At times it continues ventrally and superiorly almost to the cribriform plate of the ethmoid bone (fig. 38); however, in the majority of cases it does not extend so far, due to partial fusion between the superior border of the superior bullar fold and the attached border of the concha media (fig. 41). It is frequently also limited inferiorly and dorsally by similar fusion. Again, there may be multiple points of fusion between the superior bullar fold and the concha media, thus breaking the suprabullar recess or furrow into several compartments (fig. 43). The recess in many cases early tends to deepen or pouch laterally and inferiorly behind the bullar folds (fig. 35). In this manner the bulla becomes more or less shell-like in structure; and some of the so-called bullar cells are

thus established. The suprabullar recess is a constant point from which anterior ethmoidal cells develop (figs. 31, 34, and 43).

*The bullar furrow.* The bullar furrow is placed between the two bullar folds or accessory conchae (fig. 31). It is variable in its differentiation and not at all constant. It is generally obliterated by the superior and the inferior bullar folds becoming continuous structures in the formation of the adult bulla ethmoidalis. This coalescence is, however, not always absolute, in that an ethmoidal cell may develop from the furrow, leaving the ostium of the adult cell at the point of the primitive furrow (figs. 31 and 39). Even in many adult specimens we find evidences of this primitive furrow in the form of a shallow groove on the medial surface of the bulla ethmoidalis. An ethmoidal-cell ostium on the medial surface of the bulla ethmoidalis is almost invariably the remains of the early bullar furrow.

*The infrabullar furrow.* The infrabullar furrow is placed between the inferior bullar and the infundibular folds (fig. 31). It is very inconstant and when present is frequently obliterated by the inferior surface of the bulla ethmoidalis becoming continuous with the infundibular fold. In some cases it is fairly well marked (fig. 34) but, as a rule, it is of minor importance. Rarely an ethmoid cell develops from the furrow—the adult cell draining into the infundibulum ethmoidale.

*The infundibulum ethmoidale.* The infundibulum ethmoidale (Recessus inferior der absteigenden Schenkel der ersten Hauptfurche of Killian) is invariably present in some form. It is formed early, and obviously aids in establishing the primitive processus uncinatus. It is directed somewhat ventrosuperiorly and at its ventral and superior termination it may end blindly or develop into an anterior ethmoid cell. At other times it is variously continued into one of the frontal furrows. Rarely it continues its development ventrally and superiorly, remaining lateral to the frontal furrows, and in this way may establish the frontal sinus (figs. 36, 37, 39, and 40).

Dorsally and inferiorly it either gradually loses its depth and thus becomes continuous with the middle meatus, or it ends rather abruptly in a pocket, due to the superior and lateral curv-

ing of the dorsal end of the processus uncinatus at this point (fig. 49). From the infundibulum ethmoidale the sinus maxillaris develops, hence in the adult the latter sinus communicates with the infundibulum ethmoidale, and only indirectly via the hiatus semilunaris with the meatus nasi medius (fig. 18).

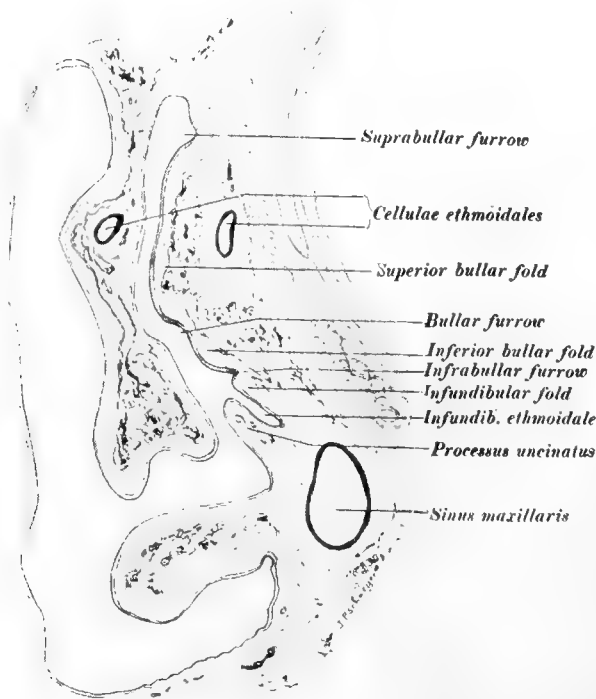


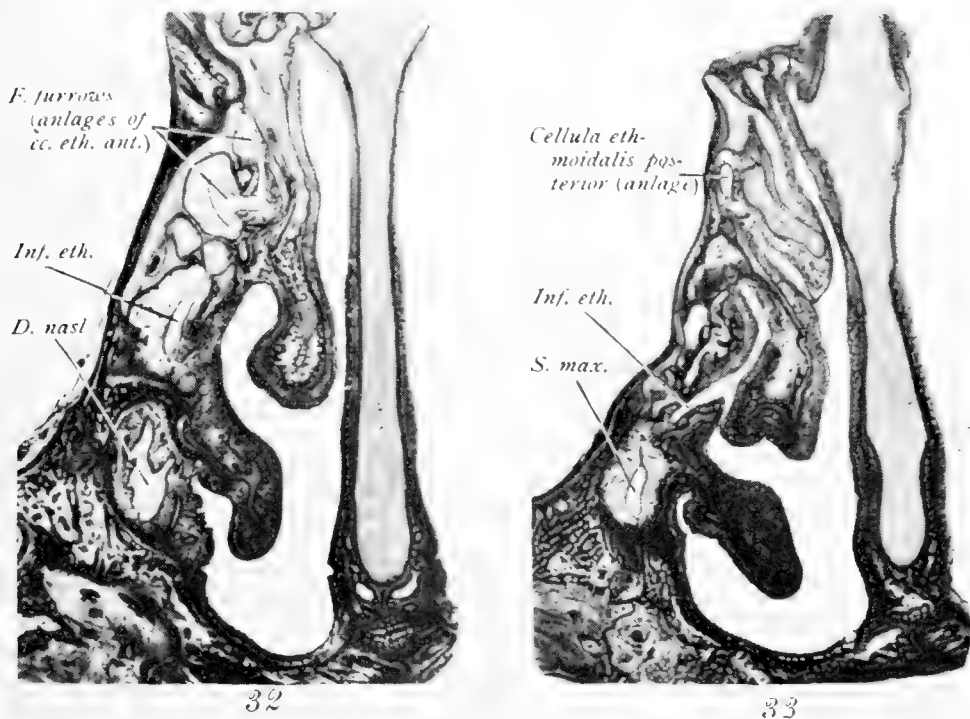
FIG. 31 ( $\times 3.9$ ) Drawing of a frontal section of the left nasal fossa of a term fetus (series D, slide 5).

Note the individual folds comprising the bulla ethmoidalis, and the fold lateral to the infundibulum ethmoidale, and the furrows that border these folds. The cell that is indicated in the superior bullar fold is an extension from the suprabullar furrow, and the cell indicated in the concha media communicates a few sections farther dorsally with the meatus nasi medius (compare with fig. 35).

*The superior bullar fold or concha.* The superior bullar fold or concha is located immediately inferior to the suprabullar recess (fig. 31). It may continue independently ventrally and superiorly almost to the cribriform plate of the ethmoid bone. In other cases it may be fused at certain points with the attached border of the concha nasalis media (figs. 41 and 43). It is frequently continuous with one or more frontal folds (fig. 41). It usually comes to form the chief bulk of the adult bulla ethmoidalis.

In many cases there is no differentiation into superior and inferior bullar folds by an intervening furrow—the bullar furrow.

*The inferior bullar fold or concha.* The inferior bullar fold or concha is, as stated above, not always differentiated from the superior bullar fold. It is, however, occasionally well isolated and stands more or less as an independent fold (figs. 34 and 39).



FIGS. 32 and 33 Photomicrographs of frontal sections through the right nasal fossa of a 7-months fetus (series B slide 31). Section fig. 32 is through the ventral portion, and section fig. 33 is through the dorsal portion of the fossa.

The frontal furrows have pouched towards the frontal region in the establishment of early anterior ethmoid cells (fig. 32).

See page 662 for explanation of lettering.

The latter condition is especially marked in the cases where an anterior ethmoid cell develops from the bullar furrow (fig. 39). I agree with Killian that the superior and inferior bullar folds (obere und mittlere Nebenmuscheln, Killian) usually coalesce to form the adult bulla ethmoidalis. Sometimes, even in the adult, we see evidences of the primitive bullar furrow, which more or less grooves the medial surface of the adult bulla. In many instances coalescence is, however, not necessary because there was at no time a differentiation into two portions.

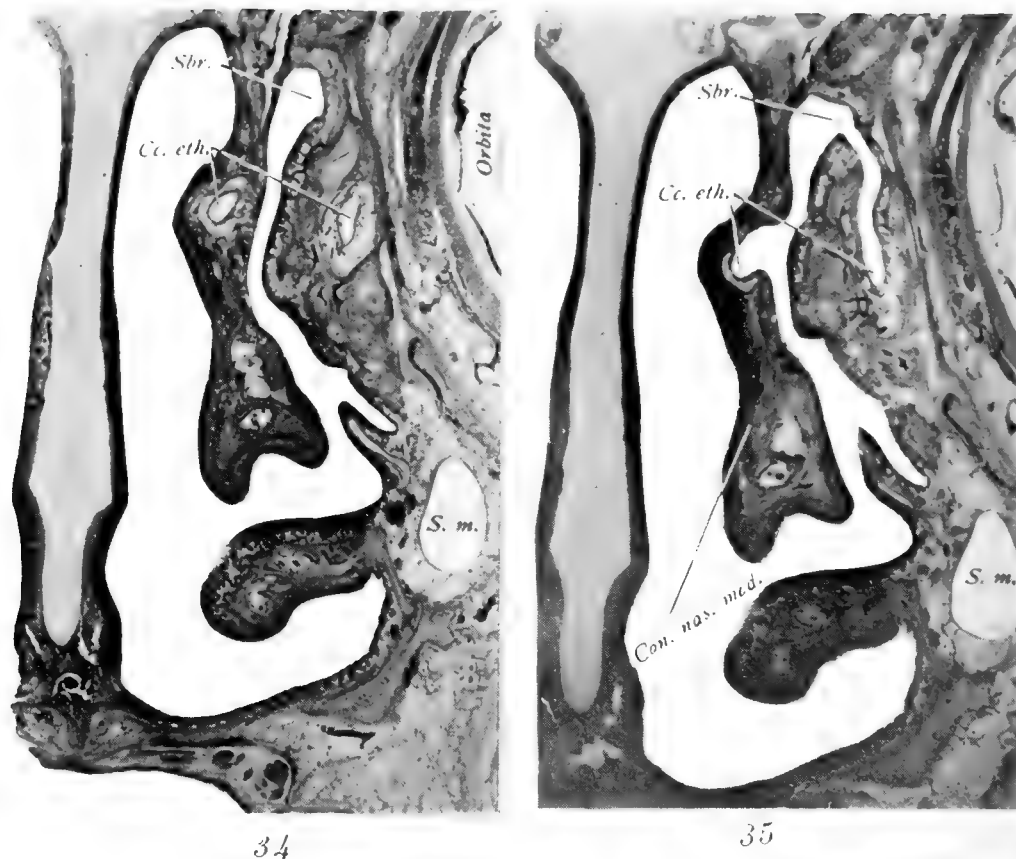


FIG. 34 and 35 Photomicrographs of frontal sections through the left nasal fossa in the region of the bullar folds, from a term fetus (series D, slides 5 and 6). Note the bullar folds, the cells in the concha media, and the superior bullar fold in fig. 34. In fig. 35, which is a section farther dorsal, the ostia of the cells are shown. It shows how the suprabullar furrow or recess tends to develop or pouch behind and lateral to the bullar folds; this in time causes the bulla to become shell-like.

*F. furrows*, = frontal furrows; *cc. eth. ant.*, = cellulae ethmoidales anterior; *Inf. eth.*, = infundibulum ethmoidale; *D. nasal.*, = ductus nasolacrimalis; *S. max.*, = sinus maxillaris; *Sbr.*, = suprabullar recess or furrow; *Cc. eth.*, = cellulae ethmoidales; *S. m.*, = sinus maxillaris; *Con. nas. med.*, = concha nasalis media.

*The infundibular fold or concha.* The infundibular fold is very rudimentary and more or less inconstant. It is located lateral to the infundibulum ethmoidale and in part forms its lateral wall (fig. 35). It is more or less separated from the inferior bullar fold by the shallow infrabullar furrow. It usually loses its identity in the adult, in that it imperceptibly passes to the bullar surface by the obliteration of the infrabullar furrow. Occa-

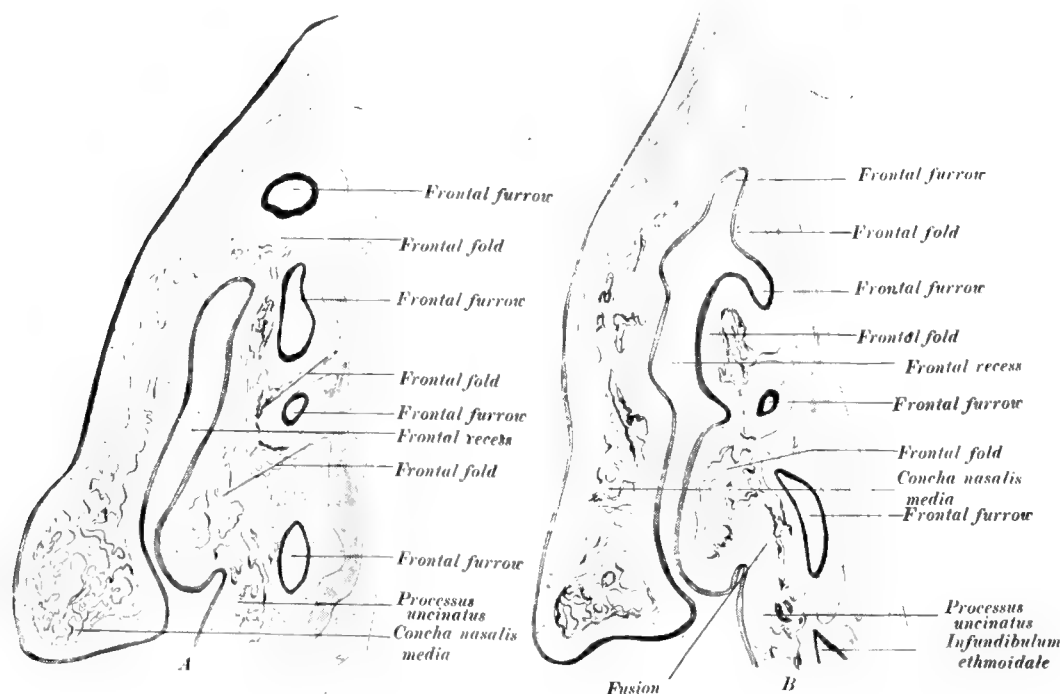


FIG. 36 ( $\times 8$ ) Drawings of frontal sections through a portion of the lateral nasal wall of a 7-months fetus, in the region of the frontal recess (series C, slide 5). Section A is farther ventral than is section B.

Note the blind ventral extremities of the frontal furrows in section A which, at this plane of section, are in reality early *anterior ethmoidal cells*. Any one of these cells may develop into a frontal sinus, or two may develop sufficiently far to be called frontal sinuses. The frontal recess may also develop into the frontal sinus.

In section B some of the furrows communicate freely with the frontal recess, and at this plane of section are more truly frontal furrows.

sionally it is well marked and is more or less isolated from the inferior bullar fold by a relatively deep infrabullar furrow. Rarely it retains its identity in the adult—this is especially so when an anterior ethmoidal cell develops from the infrabullar furrow.

*The processus uncinatus.* The processus uncinatus is a constant structure, and is medial and inferior to the infundibulum ethmoidale. As was stated before, it is the first of the accessory or hidden conchae to be differentiated. At its ventral and superior end it terminates in various ways. In some cases it is continuous with one or more frontal folds and at the same time its base continued on to the agger nasi (fig. 41). In other instances it is fused with the lateral surface of the concha media at its ventral

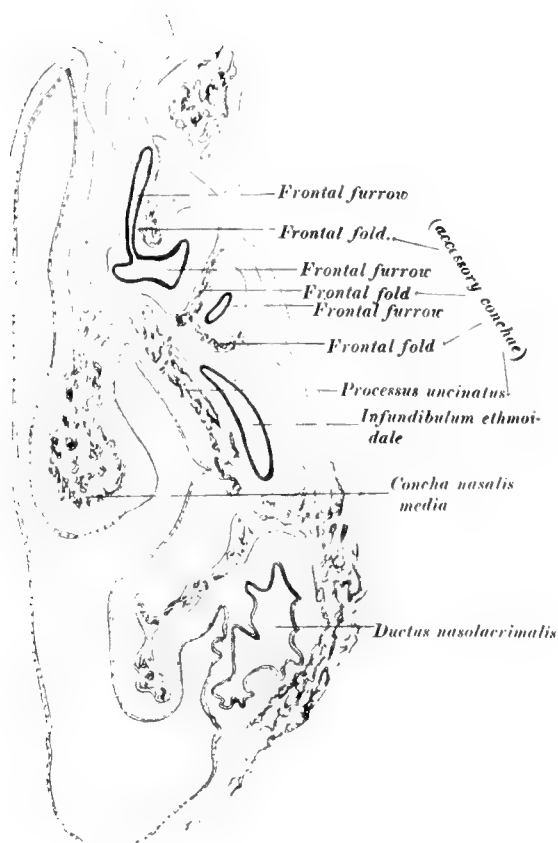


FIG. 37 ( $\times 5$ ) Drawing of a frontal section through the left nasal fossa of a fetus aged approximately 7 months (series B, slide 31). The section is in the region of the frontal recess.

Note the frontal folds (accessory conchae) and the frontal furrows. Some of the furrows, either by coalescence of the folds or by the extension of the furrows towards the frontal region, end blindly and represent early anterior ethmoid cells. The frontal recess is really obliterated by the coalescence of some of the frontal folds with the lateral surface of the concha nasalis media—compare this condition with that found in fig. 36 B.

extremity, or even fused with the ventral extremity of the bullar folds. Ventrally and inferiorly the base of the processus uncinatus becomes continuous with the surface of the agger nasi. Some observers class the processus uncinatus with the general ethmoidal conchae.

*The accessory folds and furrows of the ascending ramus of the meatus nasi medius, i.e., of the recessus frontalis.* In a previous paragraph mention was made of the rather early beginning of an extension of the meatus nasi medius from its ventral and superior part. This extension of the middle meatus towards the frontal region is the first step in the formation of the frontal sinus and certain of the anterior ethmoid cells. To this extension or recess Killian has given the appropriate name, 'Recessus frontalis.' For some time the lateral wall of this recess (ascending ramus of the middle meatus) is even and unbroken. If we examine frontal and horizontal sections of the recess of a 4-months fetus we will find the lateral plate of cartilage thickened at certain points, in the form of projections directed towards the lumen of the nasal fossa. For some time this condition prevails and the mucous membrane is not at first thrown into relief. These thickenings, which I find vary in number, are in anticipation of the folds (accessory conchae) which are present on the lateral wall of the frontal recess of a later fetus.

The folds as found in the late fetus are variable in number and as a rule are not very prominent. Their prominence depends largely upon the degree of development of the intervening furrows or pits. The folds as a rule more or less lose their identity after birth, and the furrows or pits variously remain as ostia of anterior ethmoid cells. The folds have been appropriately termed frontal folds or conchae, and the bordering furrows or pits, frontal furrows.

The frontal folds and furrows vary in degree of development and differentiation. We frequently find specimens with four well formed furrows and three resulting folds (fig. 41). In other cases, either by earlier coalescence or, I think better, by a lessened degree of differentiation, we have a smaller number of



folds and furrows (fig. 40). In fig. 41 it will be noticed that the processus uncinatus is continuous with the first and second frontal folds. The processus uncinatus in the latter case also sends a fork towards the agger nasi, and gains slight fusion with the lateral surface of the concha nasalis media. The superior bullar fold is in part directly continuous with what might be termed the third frontal fold. In fig. 38 we find that the processus uncinatus is continued ventrally and superiorly to the agger nasi; in part fusing with the concha nasalis media at this point. The superior and inferior bullar folds in the latter instance are continued superiorly to become continuous with the first and second frontal folds. Note that the third frontal furrow is more or less continuous with the suprabullar recess, and that the latter recess continues almost to the cribriform plate of the ethmoid bone. The infundibulum ethmoidale continues ventrally and superiorly into a frontal furrow. Compare this condition of the infundibulum ethmoidale with that found in figs. 39 and 41.

In fig. 40 there is only one frontal fold or concha differentiated. The concha is bordered by two frontal furrows, and the infundibulum ethmoidale is continued ventrally and superiorly into these furrows. In fig. 39 the processus uncinatus is continued ventrally on the lateral wall of the frontal recess and apparently the frontal folds extend from it. In the latter figure note also the relations of the infundibulum ethmoidale in the region of the frontal recess, and the superior and inferior bullar folds and the infundibular fold.

At times the frontal folds or conchae fuse with the lateral surface of the concha nasalis media and in this manner we have the obliteration of the frontal recess. In such cases the sinus frontalis must develop from an anterior ethmoid cell, and not by direct extension of the frontal recess (fig. 44). In fig. 36 the frontal folds have not fused with the lateral surface of the concha nasalis media and, therefore, the frontal recess is maintained. In such a condition the sinus frontalis may develop either from the frontal recess or from one or more anterior ethmoid cells. It is difficult to say in the latter figure whether the frontal folds or

conchae have coalesced with one another, thus constricting off small blind pouches (early anterior ethmoid cells), or whether the frontal furrows in anticipation of anterior ethmoid cells have pouched toward the frontal region, thus closely simulating coalescence between the several frontal folds, but making coalescence only apparent rather than real.

A comparison of figs. 36 to 42 (showing both frontal sections and surface views of the frontal recess and the descending ramus of the meatus medius) will materially aid in clearing up the various adult conditions one meets in these regions, in connection with the gross anatomy and relations of the nasofrontal duct, the infundibulum ethmoidale, and the bulla ethmoidalis.

*The accessory fold or concha of the meatus nasi superior.* At this juncture mention must again be made of a rather frequent accessory concha that is differentiated rather early on the lateral wall of the descending ramus of the meatus nasi superior (fig. 45). Seydel directs attention to it in the following words: "Bei menschlichen Embryonen fand ich einige Male in dem Spalt zwischen der mittleren und oberen Muschel, also an der Stelle, wo bei den Halbaffen die zweite Nebenmuschel liegt, eine niedrige leistenförmige Erhebung der seitlichen Nasenwand . . . Ich möchte diese Bildung als Rest der Nebenmuschel deuten."

The anlage of this accessory concha is indicated rather early, and by the fourth month of fetal life it is well established (fig. 19). Killian considers this fold important as a point for orientation: "Die Nebenmuschel im Bereiche der zweiten Hauptfurche ist, wenn nachweisbar, ein vorzügliches Orientierungsmittel namentlich zur Bestimmung des Crus descendens 3," i.e., the crus descendens of my concha nasalis superior. Some earlier writers thought that the concha superior developed or became differentiated from the concha media—this because of the furrow that is very frequently found on the medial surface of the descending crus of the concha media in the fetus (fig. 22). Seydel later pointed out the error of this contention, and Zuckerkandl agreed that he previously was in error in saying that the 'mit-

tlere Siebbeinmuschel' (concha superior) was differentiated from the 'untere Siebbeinmuschel' (concha media). Zuckerkandl then advances the theory "dass die mittlere Siebbeinmuschel nicht nur aus einer Teilung der Concha ethmoidalis inferior hervorgeht, sondern über derselben und unabhängig von ihr sich entwickelt und als Rudiment in der unteren Siebbeinspalte steckend angetroffen wird." It appears that Zuckerkandl in speaking of the variations of the 'mittlere Siebbeinmuschel,' at times mistakes the *accessory concha* of the superior meatus for his 'mittlere Siebbeinmuschel' (concha nasalis superior). What he designates as the 'Anlage der mittleren Siebbeinmuschel,' (Tafel VII, Fig. XI, Normale und pathologische Anatomie der Nasenhöhle und ihrer pneumatischen Anhänge, Bd. I, Wien und Leipzig, 1893) certainly corresponds to my accessory concha of the superior meatus, and not to my concha nasalis superior.

In case the accessory concha of the superior meatus is well developed we have fairly well formed superior and inferior recesses. The inferior recess is especially deep in the cases where the 'crista suprema' of Killian is well developed (fig. 45). This condition makes the superior meatus look much like the middle meatus, *i.e.*, the accessory concha of the superior meatus takes the place of the bulla ethmoidalis (accessory concha of the middle meatus), and the 'crista suprema' takes the place of the processus uncinatus (compare figs. 18 and 45).

The inferior recess of the superior meatus may continue superiorly and ventrally into the blind superior termination of the superior meatus. The accessory concha is, however, at times wholly or in part coalesced with the concha media, thus obliterating wholly or partly the inferior recess. Frequently a posterior ethmoidal cell develops from the inferior recess. The superior recess is often obliterated by coalescence between the accessory concha and the concha superior. In other instances the superior recess may be continued ventrally and superiorly to the blind end of the meatus superior. Occasionally an ethmoid cell develops from this recess.

## IV THE ANLAGES OF THE SINUS PARANASALES

After the preceding consideration of the meatus nasi and the conchae nasales, and the accessory folds and furrows, the genesis of the sinus paranasales becomes much simplified and fairly easy of interpretation. That all of the paranasal chambers develop from preformed or preëxisting furrows is certainly in accord with my observations. Since the paranasal or accessory cavities develop from preformed furrows and recesses, it is difficult to say just when they begin to establish anlages. I, however, believe that anlages are established much earlier than is generally supposed—in fact the furrows and recesses from which the paranasal sinuses develop are in a sense the 'primitive' anlages of these chambers. The early tendency for the sinuses to establish their 'first' anlages may be no mean factor in making the recesses and furrows what they early are.

The preëxisting spaces from which paranasal air chambers may develop, according to my studies, are: (1) the suprabullar recess, (2) the bullar furrow, (3) the infrabullar furrow, (4) the infundibulum ethmoidale, of the descending ramus of the meatus nasi medius; (5) the frontal furrows, (6) the frontal recess, of the ascending ramus of the meatus nasi medius; (7) the ventral and superior extremity of the meatus nasi superior; (8) the recessus superior, (9) the recessus inferior, of the meatus nasi superior; (10) the meatus nasi suprema I.

Of the above named spaces we rarely find air cells developing from the infrabullar furrow, and only occasionally from the bullar furrow. I, however, find that rather frequently posterior ethmoid cells develop from the inferior and superior recesses of the meatus nasi superior, and about 75 per cent of specimens in which the meatus suprema I persists we find a posterior ethmoidal cell developing from the latter meatus (the meatus nasi suprema I is present in about 62.5 per cent of adult specimens). I find that the remainder of the aforementioned spaces are quite constant in cell development. We must, however, remember that the development of cells from the frontal furrows and recess is very variable.

Because the anlagen of the paranasal chambers have primitively different relations, Killian divides them thus:

- "1. solche, die zwischen je zwei Hauptmuscheln (I. Ordnung);
2. solche, die zwischen einer Haupt- und einer Nebenmuschel (II. Ordnung) und
3. solche, die zwischen je zwei Nebenmuscheln gelegen sind (III. Ordnung)."

Varying views have been advanced by different writers as to the genesis of the paranasal chambers. Dursy thought that the establishment of all the accessory air spaces of the nose, with the exception of the anlagen of the maxillary and sphenoidal sinuses, was wholly dependent upon resorptive processes taking place in the cartilaginous and bony framework of the nose. This is certainly not the earliest factor involved in the establishment of anlagen. Others, who worked this region in some of the lower forms, considered the space included by the lateral curling of some of the conchae as ethmoidal cells—a view that is not tenable. Seydel in a subsequent paper, comes nearer the truth when he writes: ". . . ihre Entwicklung von den Spalten zwischen je zwei (auch rudimentären) Muscheln ausgeht."

Killian in '96, in an exhaustive paper, places to my mind the genesis of the paranasal chambers on a sound basis. My observations in the main agree with his. Whether, as Killian's schemata suggest, coalescence takes place between neighboring folds and conchae, thus constricting off a portion of the respective furrow to become the anlage of a cell, seems to me a difficult problem to solve. I rather hold that the nasal mucous membrane in the position of the furrow pouches in the direction of a future cell—this closely simulating coalescence of neighboring accessory folds, or an accessory fold and a concha at these points. There seems to be an inherent tendency for the nasal cavity early to enlarge its surface area by the formation of furrows, folds, and pouches. It indeed seems difficult to decide whether, for example, in figs. 36 and 44, the frontal folds or conchae coalesced with each other, thus forming blindly ending furrows, or whether the frontal furrows pouched in the frontal direction, thus simulating coalescence but making it only apparent rather than real. It,

## EXPLANATION OF FIGURES

FIGS. 38, 39, 40 Drawings from dissections of specimens from the anatomical series, Cornell University. The dissections have been made with special reference to the furrows and folds on the lateral surface of the frontal recess, and the bullar folds on the lateral wall of the descending ramus of the meatus medius. The specimens are from term fetuses.

FIG. 38 ( $\times 1.8$ ) The concha media has been partly cut away so as to expose the frontal recess and its structures and the bullar folds and furrows. The processus uncinatus has also been partly cut away so as to get a better exposure of the bullar folds and the primitive sinus maxillaris.

FIG. 39 ( $\times 1.2$ ) The concha media has been partly cut away thus securing a good exposure of the frontal recess with its folds and furrows, and the folds and furrows of the bulla ethmoidalis. The infundibular fold is also well shown in the drawing.

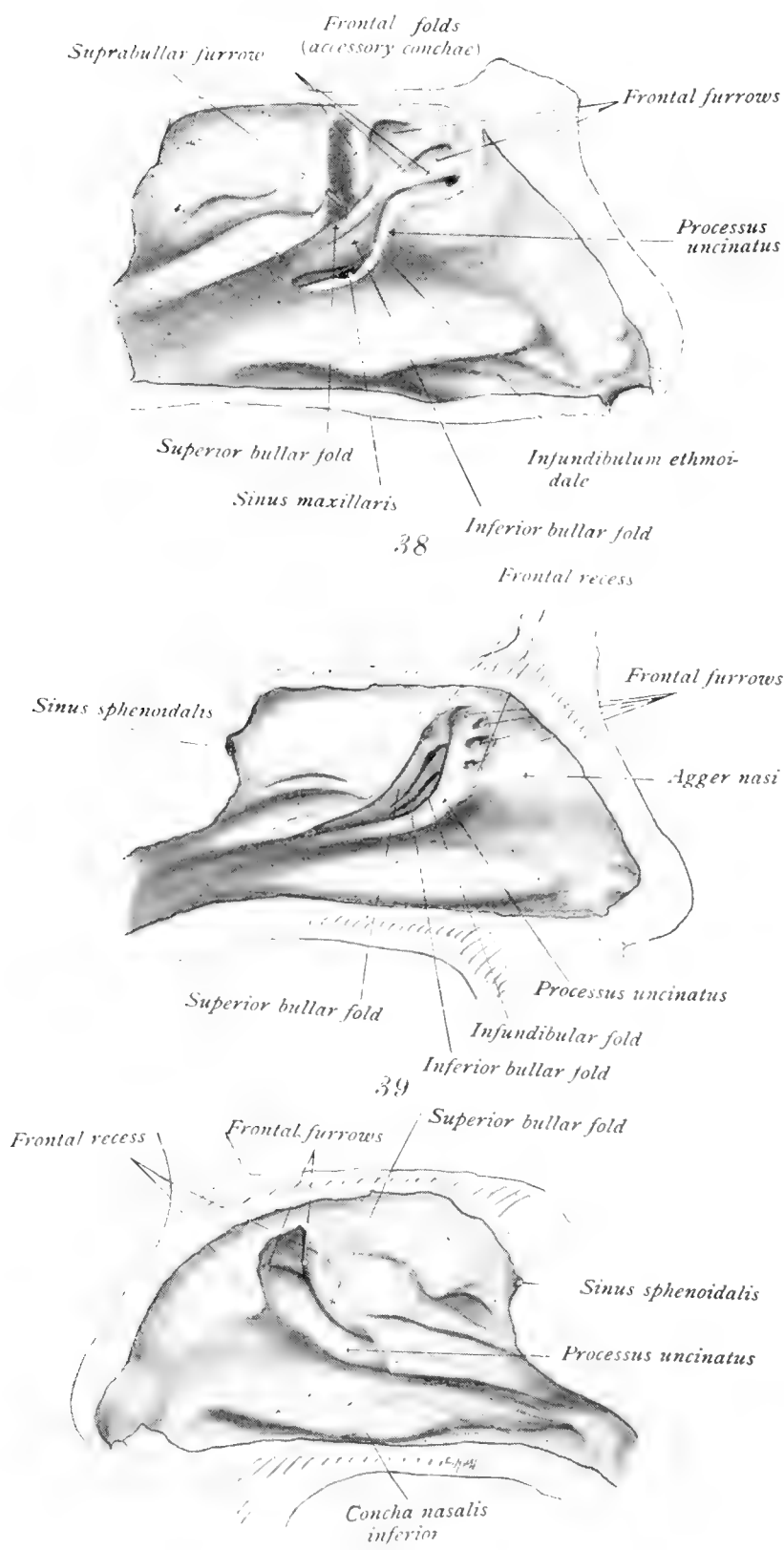
FIG. 40 ( $\times 1.2$ ) Especially note the frontal recess and the low degree of differentiation of frontal folds as compared with the specimens represented in figs. 38 and 39. The processus uncinatus is too large to allow the inferior bullar fold to be seen. Portions of the concha media and superior have been cut away.

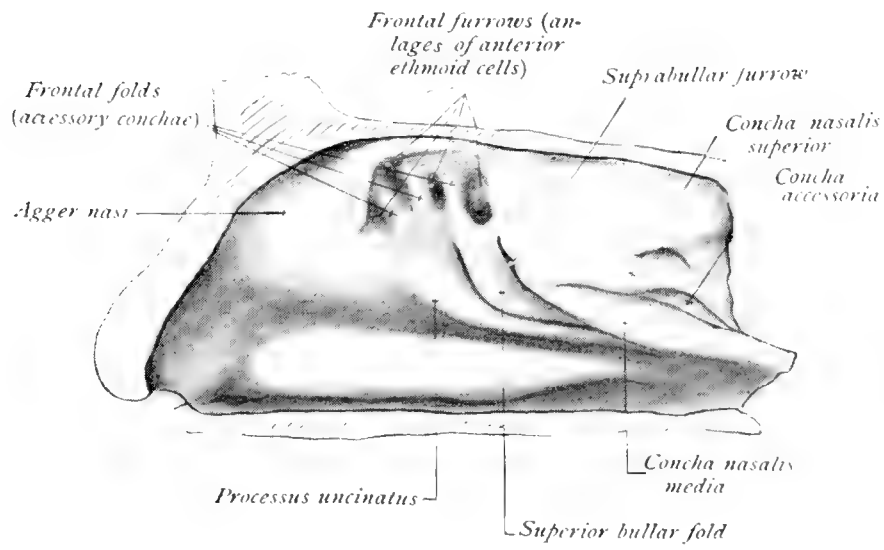
FIGS. 41, 42, 43 Drawings from dissections of specimens from the anatomical series, Cornell University. The dissections have been made with especial reference to the ascending and descending rami of the meatus medius. The concha media is partly cut away in all the specimens.

FIG. 41 ( $\times 1.2$ ) Shows the well formed frontal folds (conchae) and furrows on the lateral wall of the frontal recess, also the accessory concha of the meatus superior. From a term fetus.

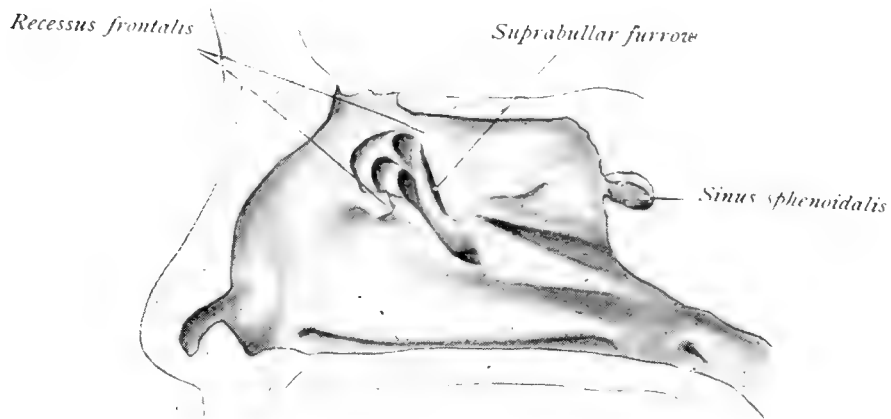
FIG. 42 ( $\times 1.2$ ) Note the manner of pouching of the frontal furrows and the frontal recess in the formation of anterior ethmoidal cells and the frontal sinus; also the developing sinus sphenoidalis. This specimen was from a child aged approximately five months.

FIG. 43 ( $\times .6$ ) In this specimen there were apparently no frontal folds nor furrows, or they were early obliterated. It will be noticed that the frontal recess is expanding towards the frontal region in the establishment of the frontal sinus. Note also the multiple ethmoid-cell anlagen in the region of the suprabullar furrow. From a child aged approximately fourteen months.

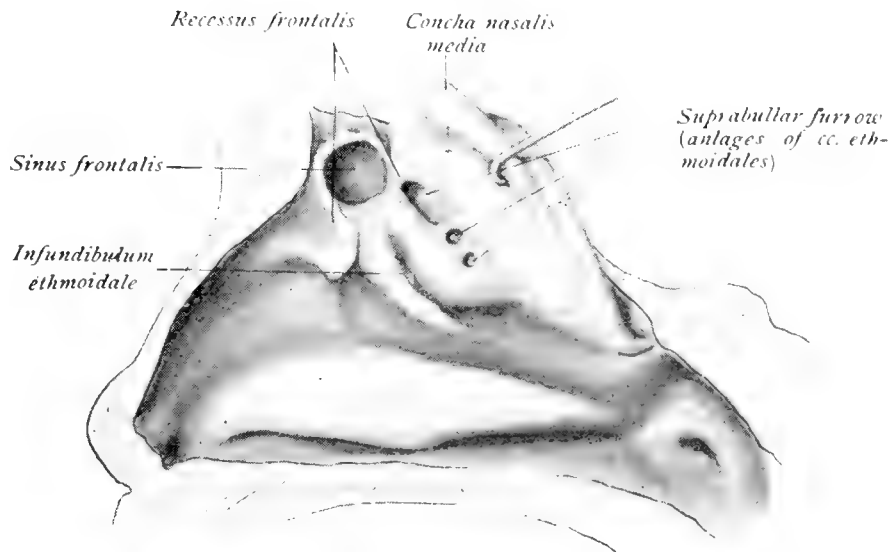




41



42



43



however, matters very little what interpretation we place here—probably both factors are more or less involved—the fact remains that from these preëxisting furrows and recesses the accessory air spaces develop. This is true of all the paranasal chambers with the single exception of the sphenoidal sinus, which is *at first*, as former writers have pointed out, nothing but a constriction from the dorsal and superior portion of the nasal fossa. Of course the factors of growth ( of the sac) and resorption (of surrounding tissue) are early brought into play (fig. 20).

### *The sinus maxillaris*

The sinus maxillaris is primitively merely a pouching or evagination of the mucous membrane of the infundibulum ethmoidale. This pouching is very evident about the seventieth day of fetal life, and is best shown by reconstructing the infundibulum ethmoidale. The earliest changes in its anlage-formation are so slight that they are difficult of appreciation by an examination of serial sections only. The anlage of the sinus maxillaris is usually represented by a single pouch, but, as I stated in a previous paper, we sometimes find two pouches growing side by side from the infundibulum ethmoidale. Again the pouching may be very extensive, and in these cases it is indeed hard to say where the infundibulum ethmoidale ends and where the anlage of the sinus maxillaris begins. In such cases the infundibulum ethmoidale is in a sense a part of the sinus maxillaris. Seydel makes the general statement that “der Raum des Infundibulum, der zwischen Processus uncinatus und Orbitalwand liegt, ist als ein Theil des Sinus (sinus maxillaris) zu beurtheilen.” If we accept the above interpretation then the sinus-maxillaris *anlage* is established with the first evidences of the infundibulum ethmoidale. Zuckerkandl also, presumably, considers the infundibulum ethmoidale a part of the early sinus maxillaris. What he labels “S.m.” (Sinus maxillaris), (Tafel XI, Figs. 5, 6, Normale und pathologische Anatomie der Nasenhöhle und ihrer pneumatischen Anhänge, Bd. I, Wien und Leipzig, 1893) is certainly in part infundibulum ethmoidale. We seldom see the primitive

sinus maxillaris so far ventral as Zuckerkandl represents it to be in Fig. 6, Taf. XI, of the above named work. What he labels "S. m." in the latter figure is, I think, wholly infundibulum ethmoidale, *i.e.*, a frontal section of the superior and ventral portion of it. I, however, think that we should speak of the sinus maxillaris as developing from the preëxisting furrow (infundibulum ethmoidale), and not consider the latter a part of the sinus maxillaris. However, as stated before, it is difficult at times to draw this distinction; especially so when the primitive sinus maxillaris is extensive and occupies the greater portion of the infundibulum ethmoidale in its early pouching.

The duplication of the primitive maxillary pouch, and the extensive pouching that we occasionally have, is entirely in accord with adult conditions—in that the adult ostium maxillare varies from a small aperture to a long slit-like opening, while in other cases the ostium is duplicated. In this connection I may be permitted to quote briefly from an earlier paper: "I have found the primitive maxillary pouch duplicated . . . This may explain some of the duplications of the ostium maxillare of the adult sinus. The two primary pouches may fuse distally, leaving the two points of evagination as the ostia maxillaria of the adult cavity. This embryonal condition in all probability explains some of the cases in which the sinus maxillaris is divided into two partly or wholly separate compartments by a vertical partition, *i.e.*, each pouch developing into an adult cavity independent of its mate . . . The great differences in the dimensions of the adult ostium may be due to the coalescence of two or more maxillary pouches; or the primitive pouching may have been single but extensive."

The early sinus maxillaris is for a time a slit-like cavity in the lateral wall of the nose. It extends inferiorly into the recess formed by the union of the cartilage of the lateral wall with that of the concha nasalis inferior (fig. 18). By resorption (of cartilage) and growth (of the sac), the sinus ultimately breaks through the cartilage in the position mentioned above (figs. 31 and 34). Its further extension into the body of the maxilla is accomplished by the simultaneous growth of the sinus and the

resorption of surrounding bone, this taking place *pari passu* with the growth of the face.

For a further consideration of the sinus maxillaris the reader is referred to a previous paper bearing directly upon this cavity.

### *The sinus frontalis*

The point from which the sinus frontalis develops is somewhat variable. Like the sinus maxillaris it develops from a preformed cavity or space. In a previous paragraph mention was made of the frontal recess, and of the folds and furrows which configure its lateral wall. The recess and the furrows especially concern us in connection with the development of the sinus frontalis. It will be recalled that the number of furrows is variable, and that the infundibulum ethmoidale bears inconstant relations to these furrows (figs. 38 to 43). At term, or even somewhat earlier, the furrows have already pouched at their superior ends towards the frontal region, thus forming early anterior ethmoidal cells. Frontal and horizontal sections will, therefore, if made at appropriate planes, show cross-sections of cavities instead of furrows (compare figs. 36, 44, and 32). The frontal recess is at this time also quite roomy and is more or less variable in its extent (figs. 36 and 41).

As Killian has properly pointed out, the frontal sinus may develop from one or more anterior ethmoid cells, from an ethmoid cell and the frontal recess, or by direct extension of the whole frontal recess. At birth it can hardly be said from what point the frontal sinus will ultimately develop into the adult cavity. According to my studies the frontal sinus may also in exceptional cases develop from the superior and ventral end of the infundibulum ethmoidale, *i.e.*, the infundibulum ethmoidale continuing its development superiorly and ventrally, lateral to the frontal recess and frontal furrows, and then expanding into the sinus frontalis. In the vast majority of cases, however, the infundibulum ethmoidale has absolutely nothing to do with the development of the frontal sinus.

Does the embryology account for the varying adult relations of the nasofrontal duct and the sinus frontalis, with the structures in the ventral and superior portion of the meatus nasi medius? To this question I must give an affirmative answer, because I think that careful analyses of this region in a series of adult specimens justifies our embryological conclusions.

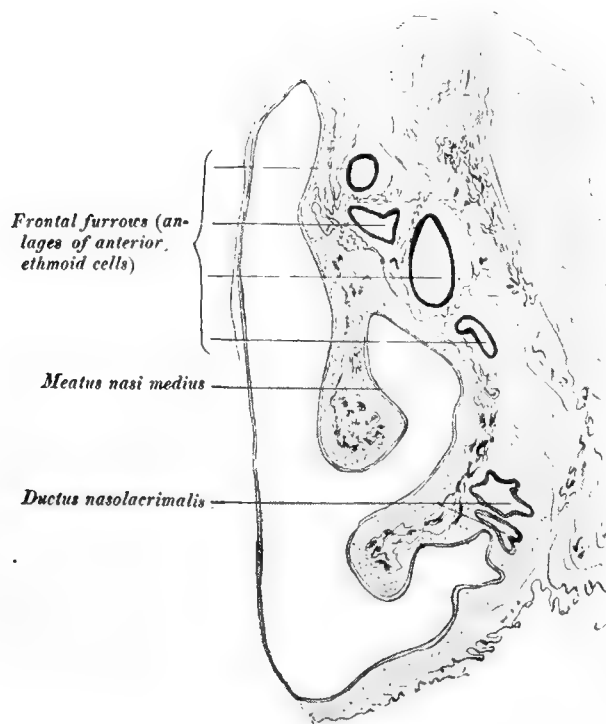


FIG. 44 ( $\times 3.9$ ) Drawing of a frontal section through the nose of a term fetus (series D, slide 4). The frontal furrows have pouched towards the frontal region and have established early anterior ethmoid cells. The frontal recess is obliterated by the coalescence of the frontal folds with the lateral surface of the concha nasalis media—compare with fig. 36 B.

It will doubtless aid in making the adult conditions one meets more comprehensible if we here refer to specific fetal conditions. In fig. 41 the infundibulum ethmoidale is in line with the third frontal furrow, but not directly continuous with it. If in this case the frontal sinus should develop from the anterior ethmoid cell of the first or second frontal furrows, or from the frontal recess directly, the nasofrontal duct of the adult sinus would doubtless communicate directly with the meatus nasi medius

and not with the infundibulum ethmoidale. If, on the other hand, the frontal sinus should develop from the cell of the third frontal furrow, the nasofrontal duct would practically be continued down to the infundibulum ethmoidale, but not *directly* continuous with it, *unless the bridge of intervening tissue were resorbed* as it frequently is. A frontal sinus developing from the cell of the third frontal furrow would in all probability have a tortuous nasofrontal duct, this of course depending largely on the disposition of the other anterior ethmoid cells.

In the specimen shown in fig. 38 the infundibulum ethmoidale and the first frontal furrow are practically continuous with each other. Should the frontal sinus develop from the first frontal furrow in such a condition, the nasofrontal duct would of course be directly continuous with the infundibulum ethmoidale in the adult. We can not say, however, that in such a condition, the sinus frontalis develops from the infundibulum ethmoidale. From adult relations it would appear as if the latter interpretation were correct; embryology, however, shows the error of this contention. If in the specimen shown in fig. 41 the frontal sinus formation should take place from the cell of the second frontal furrow, the nasofrontal duct would be continued down to the infundibulum ethmoidale at an angle, but not be *directly* continuous with it. In the specimen shown in fig. 43 (from a child aged 14 months) we find the whole frontal recess extending and developing into the frontal sinus. In the latter case the adult sinus would in all probability have no true nasofrontal duct, but the sinus would open directly into the ventral and superior portion of the meatus nasi medius.

Note the possibilities of adult relations in the fetal specimens shown in figs. 39 and 40. In the specimen shown in fig. 41 the infundibulum ethmoidale might continue its development or pouching lateral to the frontal recess and frontal furrows and thus form the frontal sinus. The aforementioned conditions are met with in the adult nose, and all may be accounted for by studying the varying fetal conditions. In a general way we may say, that when the frontal sinus develops from an anterior ethmoid cell, the adult sinus will have a nasofrontal duct—the tortuosity of

the duct depending upon the cell from which the sinus developed and upon the degree of development and the disposition of the other anterior ethmoid cells. On the other hand, when the frontal sinus develops by a direct extension of the frontal recess there will in all likelihood be no true nasofrontal duct. Occasionally the adult frontal sinus has two nasofrontal ducts. This is explained by the fact that the sinus at times develops from two anterior ethmoid cells. In many instances the frontal sinus is in reality nothing other than an extensively developed anterior ethmoid cell.

*The cellulae ethmoidales*

The ethmoidal cells all develop from preformed furrows or recesses. They are nothing but extensions from some of the meatus nasi directly, or from the accessory recesses and furrows of the meatus nasi medius and superior. From the frontal furrows of the recessus frontalis, and from the furrows of the descending ramus of the meatus medius, the anterior group of ethmoid cells develop. From the ventral and superior extremity, and the superior and inferior recesses of the meatus superior; and the meatus suprema I, the posterior group of ethmoid cells develop. The anterior group have, therefore, their ostia opening inferior to the attached border of the concha nasalis media, and the posterior group have their ostia opening superior to it. The so-called middle group of ethmoidal cells are thus classed with the anterior group, and the term 'middle ethmoidal cells' is dropped. This I think is a better classification because the cells of the bulla are closely associated with the cells of the frontal recess.

The lateral masses of the ethmoid bone and its appendages, such as major and accessory conchae, are primitively solid structures. Later, however, the lateral masses become more or less honey-combed or labyrinth-like by the developing ethmoidal cells. The ethmoid cells, while primarily confined to the lateral ethmoidal masses, frequently extend beyond these limits into the concha media, the processus uncinatus, and the agger nasi. The bulla ethmoidalis is practically always shell-like due to a cell or cells. Anterior ethmoid cells may develop far into the frontal

region, and in many cases might be classed as frontal sinuses. In some adult specimens in which two large sinuses are present on the same side of the frontal region, it is difficult to say whether they are both frontal sinuses or extensively developed anterior ethmoidal cells. Developmentally, in many cases, they are ethmoid cells, and topographically they should be classed as frontal sinuses. Occasionally the posterior ethmoid cell which develops from the meatus nasi suprema I extends into the sphenoidal sinus. The superior and the supreme conchae usually become more or less shell-like in the adult due to the developing posterior ethmoid cells.

In the formation of the ethmoid labyrinth there is no uniformity. In a general way the anterior group of ethmoidal cells is ventral to the posterior group. There is, however, at times, considerable overlapping of the two groups. Each cell as it grows from a preformed furrow or recess tends more or less towards the cribriform plate of the ethmoid bone. Even though a certain cell has its anlage-point farther inferior than another cell, it may outgrow its neighbor and force the latter to progress in a direction other than to which it was primarily directed. Later the cells in the homeycombing of the lateral ethmoidal masses grow in almost any direction. Sometimes the intervening walls break down and larger single cavities are thus formed. Seydel very aptly says: " . . . die Zellen der verschiedenen Reihen stehen gewissermassen im Wettkampf mit einander, bald überwiegen die der einen, bald die der anderen Reihe. Daher verlaufen die Trennungslinien zwischen den Reihen keineswegs immer genau wie die Muschelursprünge."

#### *The sinus sphenoidalis*

Of the sinus sphenoidalis little need be said in this paper. It is primitively nothing but a constriction from the dorsal and superior portion of the nasal fossa, a fact already pointed out by Dursy. This constriction of the nasal fossa in the formation of the anlage of the sphenoidal sinus is evident fairly early, and at birth the sinus is comparatively well advanced in most instances.

Its further development depends of course, just as in the other sinuses, upon the simultaneous growth of the sac and the resorption of bone. In this manner the sphenoidal cavity becomes well established in the body of the sphenoid bone.

In the frontal section shown in fig. 20 we have represented the manner of constriction of the superior and dorsal portions of the nasal fossae in the formation of sphenoidal-sinus anlagen. Later the sinuses are represented by very shallow pits and by the 5th month of extrauterine life the sinuses are well established (fig. 42).

#### V SOME LATER DEVELOPMENTAL CHANGES ON THE LATERAL WALL AS PRESENTED IN THE ADULT NOSE

It is not necessary to consider the adult lateral nasal wall in detail since the general anatomy is so well known, and besides the descriptions of the late fetal conditions as given, would in a measure be duplicated in a detailed description of the adult lateral wall. There are, however, certain conditions and relations of great importance which develop much later, and varying opinions are entertained on some of these points. We will, therefore, in subsequent paragraphs consider the later developmental changes with especial reference to: (1) the number of ethmoidal conchae in the adult; (2) the ethmoid cells with especial reference to their ostia; (3) the ostia maxillaria; (4) the relations of the nasofrontal duct with especial reference to the manner of communication of the frontal sinus with the meatus nasi medius.

##### *The number of ethmoidal conchae in the adult*

In previous paragraphs we dwelt at some length on the number of ethmoidal conchae that are differentiated before birth. We found that the number varied from three to five; with four major conchal folds in the ethmoidal region rather common, and five not at all unusual. The concha suprema III early loses its identity in the vast majority of cases. This is also true of the concha suprema II; however it is as a rule somewhat farther



delayed. The question naturally arises: How far does the reduction in ethmoidal conchae progress? and what is the average number of conchae in the adult in a large series of specimens?

Naturally in the cases where the meatus suprema I is very shallow and short, it becomes a personal equation as to whether the shallow furrow should be considered as a meatus or merely as a groove on the medial surface of the concha superior. In one case it means an extra concha; in the other the whole ethmoidal mass, superior to the concha media is considered as the concha superior. This is doubtless the reason why writers differ so widely in their results. Again, the accessory concha in the meatus superior, when well developed, is at times erroneously considered as the concha superior. This, of course, leads to divergent results as to the number of ethmoidal conchae that are present in the adult nose.

Zuckerkindl in "120 Kopfhälften" of adults found two ethmoidal conchae in 24 of them, and three ethmoidal conchae in 96 of them. In this number he found his "mittlere Siebbeinmuschel operkulisiert" in 11 instances. According to some of his figures he has at times considered my accessory concha of the meatus superior as his 'mittlere Siebbeinmuschel.' If we subtract 11 cases from the 96 and add them to the 24, we would presumably have a better ratio for comparison with other results. The accessory concha of the meatus superior is very prominent in some adult noses, and a large number of specimens show at least a *rudimentary* accessory concha.

The fourth annual report of the committee of collective investigation of the Anatomical Society of Great Britain and Ireland, gives the following results: Out of 152 observations, 3 cases are reported with but one ethmoidal concha; 85 cases with two ethmoidal conchae; 62 cases with three ethmoidal conchae; and 2 cases with four ethmoidal conchae. This report is the result of fifteen subreports from as many different schools, with presumably a larger number of observers. It is, therefore, difficult in such a report to have uniformity in observation because, as stated before, the personal equation as to what should be considered a concha in the cases where the differentiation is slight,

is obviously great. The report shows that in a few instances the reduction in ethmoidal conchae has progressed but little. The number of times that three ethmoidal conchae are present in the adult nose is rather low in the above report, as compared with the statements of other observers. Unless one continually

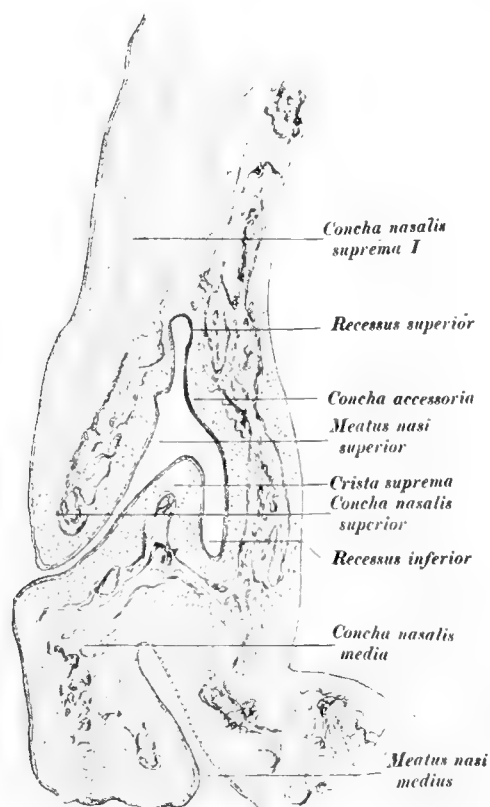


FIG. 45 ( $\times 10$ ) Drawing of a frontal section through the lateral wall of the nasal cavity of a 7-months fetus (series C, slide 8). The section is in the region of the accessory concha of the superior meatus. It shows the well developed 'crista suprema' of the concha media, also the marked curling of the latter concha.

remember the late fetal conditions, he is apt, in many cases, not to recognize the concha suprema I in the adult nose, *i.e.*, when the concha is very rudimentary.

I recently examined 120 adult lateral nasal walls, with especial reference to the number of major ethmoidal conchae. I found that 75 specimens presented three ethmoidal conchae, and the remaining 45 specimens but two ethmoidal conchae. A few of the specimens were suggestive of having four ethmoidal conchae. I

find that the ostium of the ethmoidal cell which develops from the meatus suprema I is very often a good point for orientation in determining the presence of a concha suprema I (adult).

According to Zuckerkandl's series and the series I examined, we should consider the adult ethmoidal region as usually presenting three ethmoidal conchae rather than two.

*The ethmoid cells and their ostia*

In a previous paragraph on the anlagen of the ethmoidal cells we divided them into two groups—anterior and posterior. All those cells that have their ostia opening inferior to the attached border of the concha nasalis media may be designated as anterior ethmoidal cells; and those that have their ostia opening superior to the attached border of the concha nasalis media may be designated as posterior ethmoidal cells. The anterior group are in a general way ventral to the posterior group; however there is at times considerable overlapping of the two groups.

*The posterior group of cells.* The posterior group of cells communicate with the meatus nasi superior and suprema I. The latter meatus is present, according to my specimens, in 62.5 per cent of cases; and 75 per cent of the positive specimens have a posterior ethmoid cell opening into this meatus. In some instances the cell which opens into the meatus suprema I is very large and it may encroach upon the lumen of the sphenoidal sinus. Rarely two posterior ethmoidal cells communicate with the meatus suprema I.

It is a constant condition to have posterior cells opening into the superior meatus. According to my specimens we invariably have a posterior ethmoid cell opening at the superior and ventral extremity of the superior meatus. The latter cell very frequently extends into the body of the concha media, and at times is excessively large. In previous paragraphs we spoke of an accessory concha of the superior meatus. We will recall the presence of recesses, inferior and superior to the latter concha—these recesses early giving evidence of cell-anlagen. If we carefully look over a series of adult specimens we will in many cases see evidences

of this accessory concha, which at times is well formed. Since the fetus gave evidence of developing cells from the two recesses mentioned we would in the adult expect to find ostia of posterior ethmoidal cells in these positions. Twenty-six per cent of my specimens showed a posterior cell opening in the position of the superior recess, and in 50 per cent of cases a posterior cell opened in the position of the inferior recess.

To recapitulate: The posterior ethmoidal cells open into the meatus nasi superior and suprema I. The latter meatus is present in 62.5 per cent of adult cases, and in 75 per cent of instances this meatus has a posterior ethmoidal cell communicating with it. The superior meatus has in 100 per cent of cases a cell communicating with it at its ventral and superior termination. The superior meatus has also in 26 per cent of instances a cell opening into the superior recess, and in 50 per cent of cases a cell opening into the inferior recess.

These adult conditions justify our conclusions with reference to cell-anlages in the fetus and young child.

*The anterior group of cells.* It will be recalled that we referred to anlages of anterior ethmoidal cells in both the ascending and descending rami of the meatus nasi medius, *i.e.*, in the regions of the bullar furrows and the frontal recess. If now we examine a series of adult specimens we will notice in the positions of the former cell-anlages that we now have ostia of cells. My series of specimens indicate that in 100 per cent of cases anterior ethmoid cells have their ostia in the position of the suprabullar recess or furrow. The cells that open in this position vary in number from one to three. They are directed variously behind the bulla ethmoidalis, thus causing the latter structure to be hollowed out and shell-like and at times excessively large. Some of the cells with their ostia in the position of the suprabullar recess may also be directed towards the cribriform plate or towards the frontal sinus. I find in 13 per cent of instances that there is an ostium on the medial surface of the bulla ethmoidalis, or in the position of the original bullar furrow (fig. 31). In 11 per cent of cases an ostium is found in the position of the infrabullar furrow. The

cells that communicate with the middle meatus in the location of the latter two places are usually very small.

The anterior ethmoidal cells that develop in the region of the ascending limb of the middle meatus, *i.e.*, in the region of the frontal recess, are very variable in size and position. The number of adult cells depends largely upon the degree of differentiation of frontal furrows in the fetus. Some of the cells in this position open directly into the middle meatus, others into the superior and ventral extremity of the infundibulum ethmoidale. Some also open into the suprabullar recess. Some of these anterior ethmoidal cells may extend towards the frontal sinus so that it is difficult to say whether the cavity is that of a frontal sinus or that of an anterior ethmoidal cell. The ventral and superior end of the infundibulum ethmoidale also frequently expands into an anterior ethmoidal cell, which at times is large.

#### *The conchal cells*

As was stated in a previous paragraph there is no uniformity in the development and arrangement of the cells of the ethmoid labyrinth. It will be recalled that the cells develop from pre-formed furrows, and that the former gradually extend into the lateral masses of the ethmoid bone. In this manner the lateral ethmoidal masses which were primitively solid structures become more or less labyrinth-like as age advances. The individual cells are usually separated by thin osseous plates, or as in some adult cases, merely by mucous membrane—the bony partition having been resorbed. Again the intervening walls may be entirely gone and one or the other group of cells may be replaced by a large cavity. Many of the ethmoid cells are completed by the articulation of the ethmoid with neighboring bones.

These cells in many cases are not confined to the limits of the lateral ethmoidal masses. They hollow out the superior and supreme ethmoidal conchae so that in the adult these conchae are merely thin shell-like medial boundaries of posterior ethmoidal cells. The cells also at times extend into the sphenoidal sinuses,

and also encroach upon the lumina of the frontal sinuses. Should the intervening walls break down we would have established a communication between either of the sinuses and certain of the ethmoidal cells. Ethmoid cells very frequently extend into the ventral extremity of the concha media, into the ventral extremity of the processus uncinatus, and into the agger nasi. The bulla ethmoidalis is almost invariably shell-like because of air cells. At times the concha nasalis media contains multiple air cells or it may contain a very large single cell which causes the concha to look bleb-like. It is very common for the posterior ethmoid cell which develops from the ventral and superior extremity of the meatus nasi superior to extend beyond the limits of the lateral ethmoidal mass into the concha media. Sometimes a cell develops from the so-called sinus of the concha media, which more or less hollows out the ventral extremity of the concha. Anterior ethmoidal cells may also extend into the concha media. The cells that extend into the agger nasi and processus uncinatus may communicate either with the meatus medius directly or with the superior and ventral extremity of the infundibulum ethmoidale. The concha nasalis media at times curls laterally and superiorly and thus forms the so-called sinus of the middle concha. This curling is, however, not extensive as a rule and the area included by it always opens freely into the middle meatus. It is usually unimportant.

These conchal cells have led to conflicting and some erroneous theories as to their genesis. They surely are nothing other than ethmoidal cells which have developed beyond the limits of the lateral ethmoidal masses into the appendages of these masses, *i.e.*, into the conchae. Occasionally a cell develops into the concha media which has its anlage-point on the lateral surface of this concha (figs. 34 and 35).

These conchal cells may become the seat of pathological conditions, just as do the other paranasal chambers, but the cells do not in any manner owe their origin to such conditions. For a further consideration on conchal cells the reader is referred to a previous paper bearing directly on these cells.

*The ostia maxillaria*

In this connection little need be said about the ostium maxillare. In the adult it of course is in the position of the primitive sinus maxillaris, *i.e.*, the adult ostium corresponds in position to the evagination of the mucous membrane in the infundibulum ethmoidale for the formation of the anlage of the sinus maxillaris. The formation of the ostium maxillare was, therefore, considered in previous paragraphs and nothing further need be added here. While the ostium maxillare may be primitively double, *i.e.*, the anlage of the sinus maxillaris may be represented by a double pouching, it (the ostium) at times becomes duplicated later in life. This latter duplication of the ostium needs to be considered since certain later developmental processes are involved. Very frequently there is also an ostium present in the adult nose which communicates directly between the sinus maxillaris and the meatus nasi medius. This ostium is not present in the fetus and young child, hence a later developmental process is here involved. In order to complete the consideration of the various developmental stages of the lateral nasal wall it will be necessary briefly to consider the duplication of the ostium maxillare and the ostium maxillare accessorium.

*The ostium maxillare accessorium.* In a previous paper I referred to the frequent occurrence of an accessory aperture of the sinus maxillaris. In the same article I dwelt briefly on the theories held by several writers as to the genesis of this opening, and stated that I hoped to study the accessory aperture more extensively in the fetus and child, to see whether the opening, after all, at times, did not have an embryological significance. Since then I have had the opportunity of examining a larger number of specimens of the lateral nasal wall of the adult, child, and fetus.

This accessory ostium is, as a rule, situated in the membranous portion of the lateral wall of the middle meatus, a short distance above the cephalic and attached border of the inferior nasal concha, at about the junction of its middle and posterior thirds. In some instances the accessory ostium is found immediately dorsal

to the infundibulum ethmoidale— occasionally actually extending into the latter. This accessory ostium is usually single but occasionally it is duplicated, and rarely three accessory apertures are present in this portion of the middle meatus. The aperture must not be confused with the duplication of the ostium maxillare which is located in the depth of the infundibulum ethmoidale; however at times very near its dorsal extremity.

Nathaniel Highmore, who apparently was the first anatomist to describe the maxillary sinus, does not mention anything about this accessory ostium. J. Cruveilhier refers to an orifice in the middle meatus, apparently the accessory ostium of the sinus maxillaris, which he considers the “plus ordinairement *l'orifice du sinus maxillaire*.” Further he says that “Cet orifice semble manquer quelquefois; on le trouve alors au niveau de la partie moyenne de l'infundibulum; on dirait, dans ce cas, que le sinus maxillaire communique directement avec les sinus frontaux et non avec les fosses nasales.” He also calls attention to the fact that the maxillary sinus may possess two apertures: “Il n'est pas rare de voir le sinus maxillaire communiquer à la fois et dans le méat moyen et dans l'infundibulum.”

From the above we must conclude that Cruveilhier considered the accessory aperture as the more regular ostium of the sinus maxillaris, but recognized that it is inconstant. He erroneously thought that the ostium maxillare, which is constant and communicates with the infundibulum ethmoidale, was not always present, and when it was present, that the maxillary sinus communicated with the frontal sinus and not with the middle meatus. We only rarely have *direct* communication between the frontal and maxillary sinuses (Cryer, Brophy, and Schaeffer have reported such cases). While from a *practical point of view* the frontal sinus communicates with the maxillary sinus in many cases, due to the infundibulum ethmoidale acting as a gutter between the frontal region and the ostium maxillare, we have no right to say that the two sinuses are in communication with each other *directly*. In all cases the sinus maxillaris communicates indirectly with the meatus medius by way of the hiatus semilunaris. Gosselin, however, recognized that the sinus maxillaris constantly communi-



cated with the 'Trichter' (infundibulum ethmoidale). Henle figures an accessory opening of the sinus maxillaris under the name 'unbeständige Communicationsöffnung.'

Giraldès in 100 cadavers found this accessory ostium "acht bis zehn Mal." Zuckerkandl reports it present "in jedem neunten bis zehnten Falle." Chiari and Hajek found an accessory ostium in every fifth case. Turner found it four times in nine dissections. In a former paper I reported an accessory maxillary ostium present thirty five times out of 80 specimens examined, or a percentage of 43.75. Three of the specimens presented two accessory ostia, or a percentage of 3.75.

Since the publication of the aforementioned paper my attention was directed to a report (Fourth annual report of the committee of collective investigation of the Anatomical Society of Great Britain and Ireland) which was inadvertently overlooked before. This report covers the examination of 152 specimens. In this number of specimens it was found that 53 percent of maxillary sinuses possessed but one aperture (presumably the ostium maxillare); 44.1 per cent, two apertures (presumably the regular ostium, and either the duplication of the latter or the ostium accessorium); and 2.9 per cent, three apertures (presumably two accessory ostia besides the regular aperture). The sinus maxillaris communicated with the infundibulum ethmoidale by two apertures (a duplication of the regular ostium) in 17.6 per cent of cases. In this report no mention is made as to what is considered a duplication of the normal aperture. In some cases an accessory ostium is placed immediately dorsal to the infundibulum ethmoidale, in fact is very often continuous with the infundibulum. I imagine that some of the latter cases are included among the 17.6 per cent of specimens. Unless the opening were wholly included within the dorsal end of the infundibulum I considered the aperture as an accessory ostium, communicating directly between the sinus maxillaris and the meatus nasi medius, and not as a duplication of the ostium maxillare proper. With this fact kept in mind the above report agrees closely with the figures in my former paper and also with the results obtained in the following series.

I recently examined 125 adult specimens (including those of my former series) of the lateral nasal wall and found that 53 of them had accessory ostia communicating directly between the maxillary sinus and the middle meatus—a percentage of 42.4. Three of the specimens presented two such openings—a percentage of 2.4.

J. A. Giraldès was apparently the first to consider this opening from a developmental point of view. He came to the conclusion “dass in allen Fällen, wo diese abnorme Oeffnung besteht, sie immer das Product eines pathologischen Vorganges und durch eine wirkliche Perforation zu Stande gekommen ist.” He however, considered this aperture much less common than it is, thinking it present in only 8 or 10 per cent of instances. Giraldès bases his pathological theory on the fact that he had the privilege of following the “Entwicklungsphasen von der Verdünnung der Schleimhaut des Ganges bis zur vollständigen Durchbohrung.” Zuckerkandl corroborates the thinning of the mucous membrane, but does not hold to the pathological theory. The latter author has seen some cases where an accessory aperture was caused by the gradual wearing of a “zugespitzter Hakenfortsatz der Nasenscheidewand,” which finally resulted in an opening on the lateral wall of the middle meatus.

While some accessory apertures are obviously due to a pathological process as suggested by Giraldès, and others are caused in a mechanical manner by spurs on the nasal septum, as suggested by Zuckerkandl, we certainly must look elsewhere in the vast majority of cases to find the genesis of this very common aperture.

I agree that there is a thinning of the mucous membrane in the position of the accessory ostium, but believe that the explanation for this is found in the development of the sinus maxillaris. In infancy the walls of the sinus maxillaris are comparatively very thick. We know that the sinus cavity increases by the simultaneous growth of the sac and the resorption of surrounding tissue, these processes taking place *pari passu* with the growth of the face. In this manner the sinus walls become thinner, up to a limit, as age advances. The thinning out apparently progresses unevenly, as evidenced by the very uneven walls of many

adult cavities. On the base or medial wall of the cavity we have an area that is in time composed of merely two layers of abutting mucous membrane, one the mucous membrane of the middle meatus, and the other the mucous membrane of the maxillary sinus. These two layers of mucous membrane with no intervening bone offers very little resistance to the growing maxillary cavity. In time they become so thinned out and attenuated in many instances that ultimately an opening is formed, thus establishing the ostium maxillare accessorium. This reminds one of the early thinning and attenuation and ultimate rupture of the two layers of abutting epithelium—the bucconasal membranes—in the establishment of the primitive choanae.

If we accept this view, and to me it seems plausible, as to the genesis of the ostium maxillare accessorium, we would not expect to find the ostium in fetuses nor in young children. We would neither expect to find this accessory aperture often in the young adult, *i.e.*, before the twentieth year of age. We would, however, especially look for it in the more advanced adult, after the walls of the sinus maxillaris have been thinned out by the large adult cavity. This is in accord with my results. I have never been able to find the ostium maxillare accessorium in the fetus nor in the child. It occurs, as far as my observations would indicate, only in the adult. I have found it in adults between the ages of twenty and ninety years. It is a very frequent aperture from thirty-five years on. Symington likewise never found the accessory ostium in children. He says: "In children I have never found more than one aperture, viz., that into the infundibulum."

I have, therefore, come to the conclusion that the ostium maxillare accessorium is in most instances established by the developing sinus maxillaris. The growth of the sinus causes the two layers of abutting mucous membrane to become thinned out and attenuated, ultimately resulting in an opening in very many adult noses. Some are doubtless due to a pathological process, and others produced in a mechanical manner by septal spurs. Enlarged middle conchae may also wear holes into the maxillary sinus in this position; this is, however, rare.

*The duplication of the ostium maxillare.* In a previous paragraph I spoke of the double pouching of the primitive maxillary sinus. By the distal fusion of the two pouches the points of evagination would remain as the adult ostia maxillaria, yet the adult cavity would be single. Other duplications of the regular

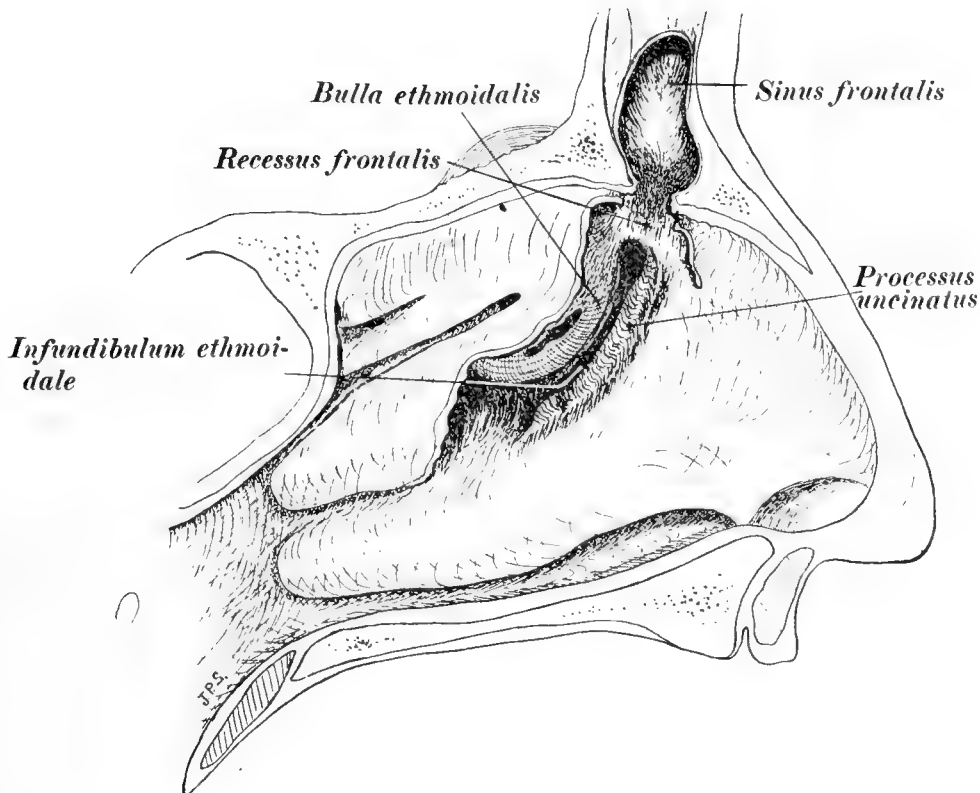


FIG. 46 A semidiagrammatic drawing of an adult lateral nasal wall. The concha nasalis media is represented as partly cut away so as to expose the underlying structures. Note the nasofrontal relations, and that there is no true nasofrontal duct. The frontal sinus presumably developed by an extension of the frontal recess directly. Compare this with the conditions represented in figs. 47, 48, and 49.

maxillary ostium are doubtless caused in a manner similar to the establishment of the ostium accessorium, *i.e.*, by the attenuation and ultimate rupture of the mucous membrane in this position. The same conditions prevail here as in the position of the ostium accessorium, since it is in the immediate neighborhood.

*The communication of the frontal sinus with the middle meatus*

We previously considered the early conditions of the frontal recess with especial reference to the frontal furrows found on its lateral wall. Reference was made to the possible points of origin of the sinus frontalis. It now remains briefly to consider the ventral and superior portion of the meatus medius with especial reference

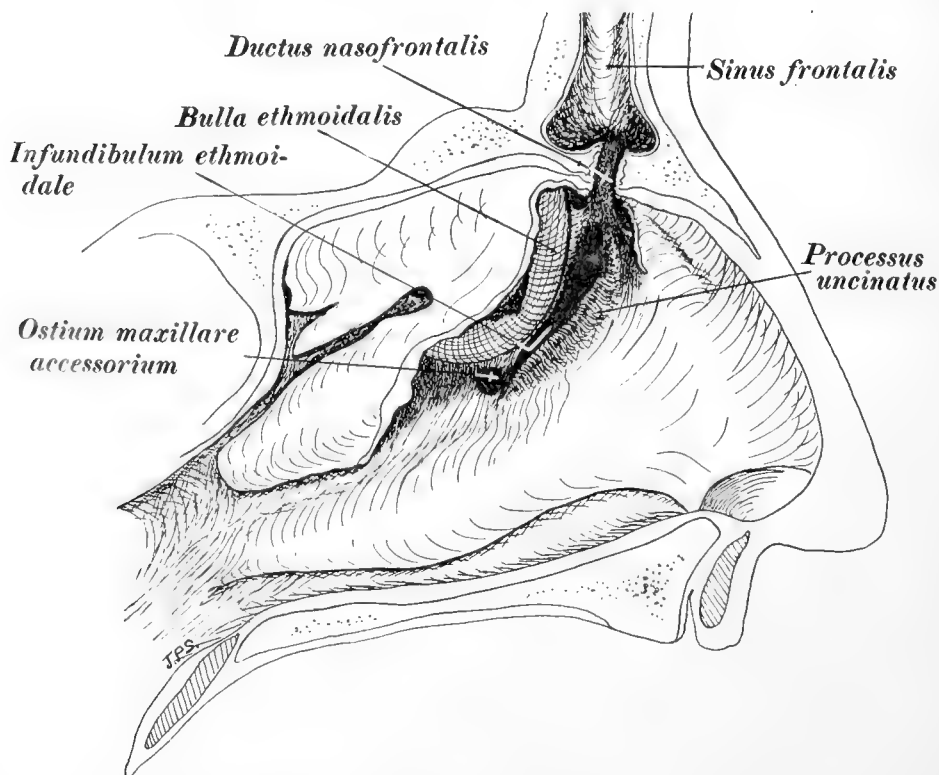


FIG. 47 A semidiagrammatic drawing of an adult lateral nasal wall. The concha nasalis media is represented as partly cut away. Compare the nasofrontal relations with those found in fig. 46. In this case there is a nasofrontal duct which meets the infundibulum ethmoidale at an angle. Some of the secretion from the frontal sinus would find its way directly into the meatus medius, and the remainder would pass into the superior and ventral portion of the infundibulum ethmoidale. The frontal sinus in this case presumably developed from an anterior ethmoid cell.

to the manner of communication of the frontal sinus with the middle meatus in the adult, to see whether our earlier conclusions on this portion of the lateral nasal wall were justifiable. We previously concluded that the frontal sinus might develop from the frontal recess directly; from one or more anterior ethmoid cells

which developed from the frontal furrows; from the recess and a cell; or rarely by direct extension of the superior and ventral extremity of the infundibulum ethmoidale. In figs. 46 to 49 we have semidiagrammatic representations of prevailing adult types of nasofrontal relations.

In fig. 46 the frontal sinus is continued down to the frontal recess, that is, to the ventral and superior portion of the meatus medius. In such a case there is no true nasofrontal duct and presumably the frontal sinus developed by a direct extension of the frontal recess. Note that the infundibulum ethmoidale ends in an air cell and that it is not continuous with the frontal sinus. Occasionally in these instances the infundibulum ethmoidale is carried much nearer to the frontal sinus, and secretion from the latter may drain almost directly into the ventral and superior portion of the infundibulum.

In fig. 47 there is a nasofrontal duct. This represents a very common condition. Note that while the infundibulum ethmoidale is not continuous with the nasofrontal duct it nevertheless meets the latter duct at an angle. Secretions from the frontal sinus would drain partly into the infundibulum ethmoidale and partly into the middle meatus directly. Doubtless the frontal sinus in such instances developed by the extension of an anterior ethmoidal cell that had its origin in one of the frontal furrows.

In fig. 48 we again have the representation of a prevailing condition or type. There is again a nasofrontal duct, but it is not in line with the infundibulum ethmoidale. Practically all secretion from the frontal sinus would drain into the meatus nasi medius. We must here conclude that the frontal sinus developed by an extension of an anterior ethmoidal cell, presumably the cell from the first frontal furrow.

In fig. 49 the infundibulum ethmoidale is continuous with the nasofrontal duct. There are two possibilities of development for the frontal sinus in these cases. In the first place the infundibulum ethmoidale may have continued its development superiorly and ventrally, lateral to the frontal furrows, and then enlarged into the frontal sinus. Another explanation may be found in the fact that the infundibulum ethmoidale may have been continu-

ous with a frontal furrow; if not continuous, the intervening lamella of tissue may have been resorbed and the two channels thus made continuous. *It is not common for the infundibulum ethmoidale to be DIRECTLY continuous with the nasofrontal duct.* At first sight it may appear so, but careful dissection will show that, in the majority of cases where intimate relations exist between the in-

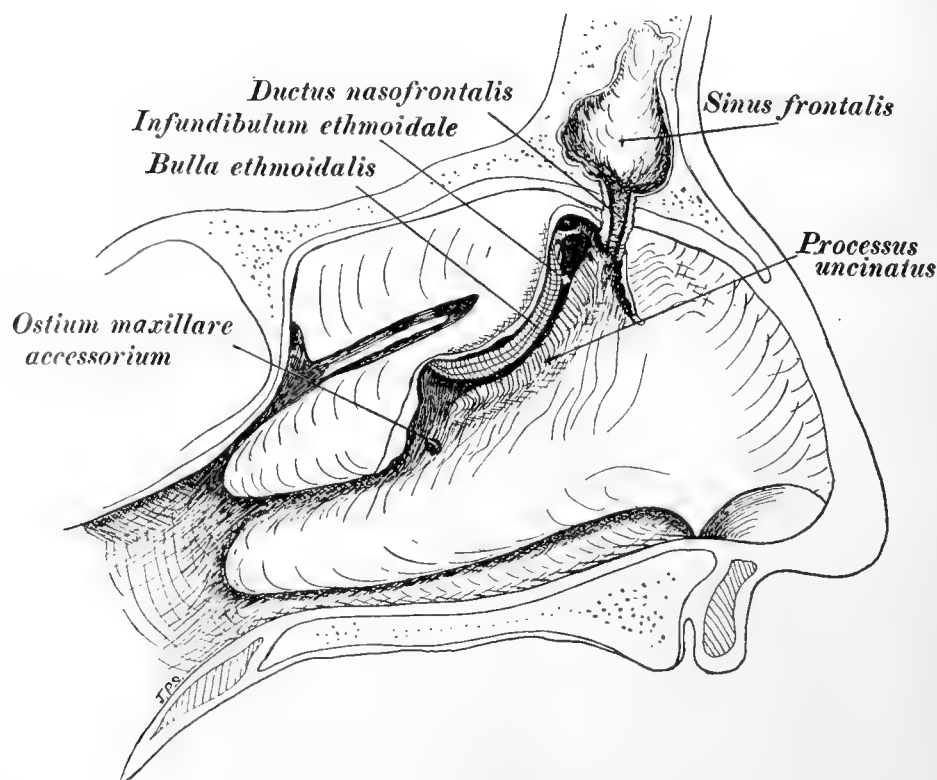


FIG. 48 A semidiagrammatic drawing of an adult lateral nasal wall. The concha media is again represented as partly cut away so as to expose the operculated structures. Compare the nasofrontal relations with those represented in figs. 46 and 47. Practically all of the secretion from the frontal sinus would drain directly into the meatus nasi medius. The frontal sinus is doubtless an extension of an anterior ethmoidal cell.

fundibulum ethmoidale and the nasofrontal duct, *the two channels meet each other at varying angles.* At times there is actually a slight groove connecting the two channels. We have, however, no right to say that in such cases, the nasofrontal duct is directly continuous with the ventral and superior end of the infundibulum ethmoidale.

In fig. 50 anterior ethmoidal cells from two frontal furrows are developed sufficiently to be called frontal sinuses. Both communicate with the frontal recess or the ventral and superior portion of the meatus medius. In such instances the intervening wall may break down and an adult frontal sinus with two nasofrontal ducts will be present. Occasionally we find a frontal sinus with two

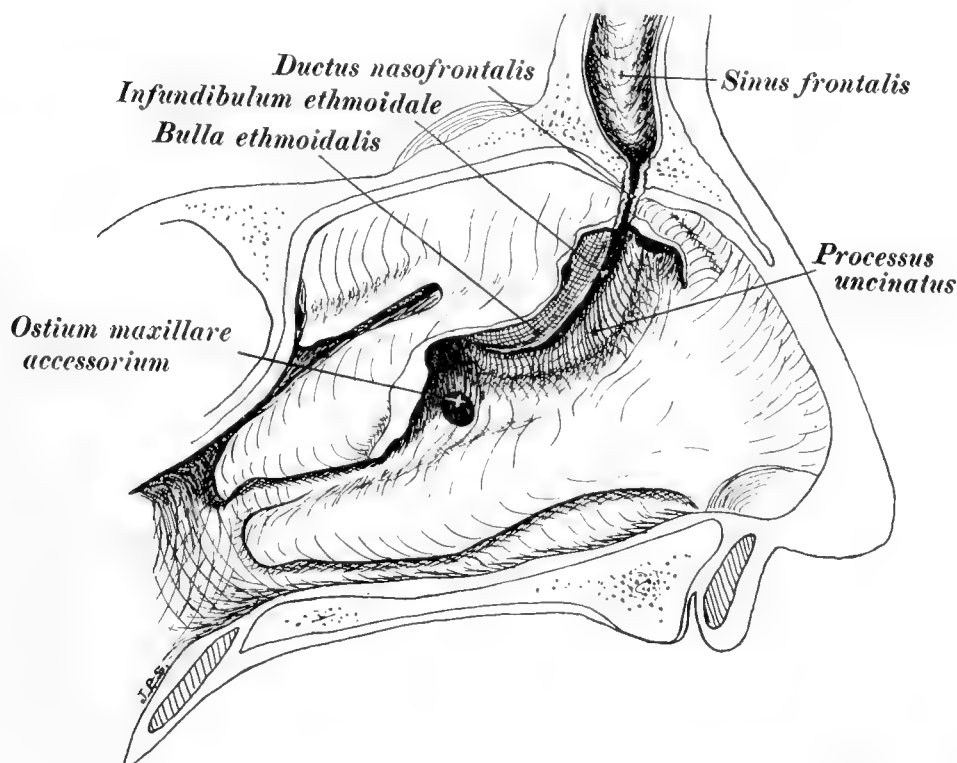


FIG. 49 A semidiagrammatic drawing of an adult lateral nasal wall. The concha nasalis media is partly cut away. The infundibulum ethmoidale is *directly* continuous with the nasofrontal duct—a condition not common.

ducts leading from it, and the above explanation for such a condition seems plausible.

If we take all the cases that fall under the types as represented by figs. 47 and 49, they will include about 56 per cent of my specimens, type, fig. 47, being much the more common of the two. In a previous paper these two types were included among the specimens as representing positive fronto-maxillary relations. The specimens falling under the type as represented by fig. 46 will also frequently fall under the above classification. The specimens



falling under the type as is represented by fig. 48 will include approximately 40 per cent of my adult specimens. The latter were in a previous paper included among the specimens as representing negative fronto-maxillary relations. Specimens will, of course, now and then differ somewhat from these types, but they will all in a general way fall under one or the other group.

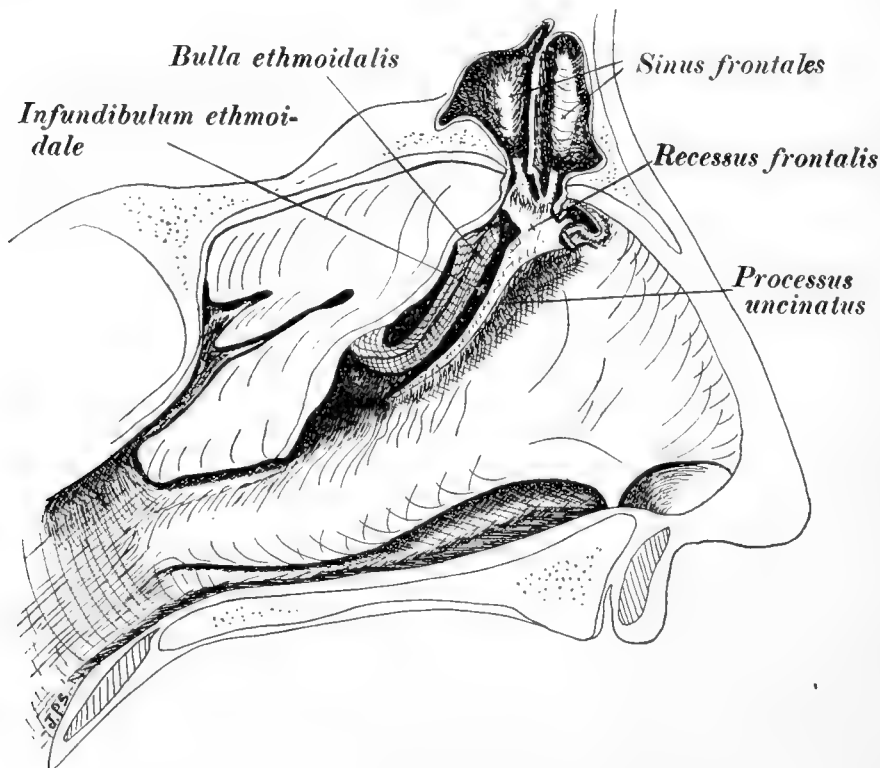


FIG. 50 A semidiagrammatic drawing of an adult lateral nasal wall. The concha media is partly cut away so as to expose for study the underlying parts. It gives evidence of three primitive frontal furrows, viz., two frontal sinuses which doubtless developed from anterior ethmoid cells, and a third cell which extends towards the agger nasi. The region of the frontal recess is well shown in the drawing.

In this manner we can account for two frontal sinuses on one side. We can also account for the so-called absence of a frontal sinus. Should the frontal recess or an anterior ethmoidal cell of a frontal furrow, stop short of the vertical portion of the frontal bone we would of course have a diminutive frontal sinus—in fact no frontal sinus as far as position is concerned. We must, however, remember that the first evidences of the frontal sinus

must not be sought in the vertical portion of the frontal bone but in the superior and ventral portion of the meatus medius, *i.e.*, in the frontal recess. The developing frontal sinus may not progress farther than the horizontal portion of the frontal bone. The different views held on the presence and absence of the frontal sinus is doubtless due to differences of opinion as to what should be called a frontal sinus, and how far the development must progress towards the frontal region before the cell has reached the dignity of a frontal sinus.

## VI SUMMARY

1. The nasal anlage establishes itself about the third week of embryonal life as localized thickenings of the ectoderm, located on both sides of the outer surface of the wall of the fore-brain, immediately superior to the primitive oral fossa.

2. During the fourth week the nasal areas become passively depressed, due to an increase in the thickness of the surrounding mesenchyme. In this manner the nasal pits become formed.

3. For some time the nasal pits communicate freely with the primitive oral fossa. The maxillary and the lateral nasal processes abut and fuse with the medial nasal processes, thus separating the nasal pits from the oral fossa.

4. For some time the lines of fusion of the maxillary and the lateral nasal processes with the medial nasal processes are represented by strands of ectodermal tissue. These ectodermal fusion-lines soon disappear ventrally and are replaced by mesenchyme; *i.e.*, the mesenchymal tissue of the maxillary and lateral nasal processes becomes continuous with that of the medial nasal processes.

5. Farther dorsally the ectodermal tissue between the fused maxillary and medial nasal processes does not wholly disappear, but strands of ectodermal tissue remain at the points of fusion. This ectodermal tissue thins out and ultimately ruptures, thus establishing the primitive choanae.

6. The nasal pits deepen dorsally and superiorly, and in a 35-day embryo the olfactory organ is represented by two blindly

ending pouches lying in the mesenchymal tissue above the oral cavity. These blind pouches may be termed the primitive nasal fossae.

7. The dorsal extension of the blind primitive nasal fossae continues until the ectoderm of the nasal fossae meets the ectoderm of the oral cavity. We have now in these positions merely thin membranes composed of two layers of abutting epithelium—nasal and oral—separating the dorsal portions of the primitive nasal fossae from the oral cavity.

8. These membranes—the *membranae buconasales*—become attenuated and thinned out, ultimately resulting in rupture. In this manner the primitive choanae are established, and again a communication between the nasal fossae and the oral cavity. The primitive choanae are established approximately from the 35th to the 38th day of embryonal life.

9. When first formed the nares communicate freely with the exterior, but shortly afterwards, in many cases, they become plugged by an overgrowth of epithelium. This plugging may be complete or more or less fenestrated in character.

10. With the formation of the primitive choanae we have also established the primitive palate, *i.e.*, the portion of the roof of the primitive oral cavity extending from the primitive choanae to the nares.

11. The palatal processes; by fusing along their opposed edges in the median plane; establish the definitive palate. In the formation of the definitive palate the nasal fossae appropriate a considerable portion of the primitive oral cavity.

12. The nasal cavity which is early divided anteriorly by the medial nasal processes, becomes divided into fossae farther dorsally by the growth and fusion of the nasal septum with the mid-palate line. This division of the nasal cavity into fossae takes place from before backwards.

13. Coincidentally with the formation of the definitive palate the primitive choanae elongate and ultimately come to occupy the position of, and thus become, the permanent choanae.

14. The lateral nasal wall is at first extremely simple, and it presents no evidence of its later complexity.

15. The first change on the lateral nasal wall from a more or less even surface is the production of very shallow grooves. The latter appearing inferior and superior to the position of the primitive concha nasalis inferior (maxillary fold). These shallow grooves at once throw into slight relief the greater portion of the lateral nasal wall—the anlage of the concha nasalis inferior.

16. Shortly after the first a second fold appears, outlining the anlage of the ethmoidal conchae.

17. From three to five ethmoidal conchae become differentiated before birth.

18. The ethmoidal conchae in a general way present ascending and descending crura. The ascending crura are, however, in many cases not well differentiated. At times the ascending crura are represented by a general ascending-crural mass, but individual ascending crura are not outlined.

19. The ethmoidal meatuses, including the meatus nasi medius, also in a general way present ascending and descending rami. The descending rami are, however, by far the most marked. In fact many ethmoidal meatuses have no ascending rami. The ascending ramus of the meatus medius is the best outlined. Occasionally the meatus superior possesses a well marked ascending ramus.

20. The integrity of the ethmoidal conchae and meatuses depends upon the descending limbs rather than upon the ascending limbs.

21. The ascending and the descending rami of the meatus medius present accessory conchae and furrows which are comparable to structures found in other mammals.

22. The descending ramus of the meatus superior also presents an accessory concha on its lateral wall. It is at times well marked, and again it may be very rudimentary or be entirely wanting.

23. In the region of the knees or bends of the ethmoidal conchae we frequently find lobules, and on these lobules secondary nodules are occasionally seen.

24. The descending ramus of the concha nasalis media very frequently presents furrows on its medial surface. One of these

furrows at times more or less divides the concha into superior and inferior portions.

25. The sinus paranasales all develop from *preformed* furrows or pits, with the single exception of the sinus sphenoidalis which is *primitively* nothing but a constriction from the dorsal and superior portion of the nasal fossa.

26. The sinus maxillaris develops by an evagination from the infundibulum ethmoidale. The primitive sinus may be duplicated, in that the pouching is occasionally double. The primitive pouching is at times extensive, including a goodly portion of the infundibulum ethmoidale. These facts doubtless account for the very large ostium maxillare of some adults and the duplication of the ostium maxillare in some cases.

27. The sinus frontalis may develop either from the frontal recess or from an anterior ethmoidal cell. It may also have a double origin, *i.e.*, from two anterior ethmoidal cells, or from an ethmoidal cell and the frontal recess. Rarely it develops by a direct extension of the infundibulum ethmoidale.

28. The anterior group of ethmoidal cells develop from the frontal furrows of the ascending ramus of the middle meatus, and from the furrows found on the lateral wall of the descending ramus of the middle meatus. The infundibulum ethmoidale at its superior and ventral termination very frequently dilates into an anterior ethmoidal cell.

29. The posterior group of ethmoidal cells develop from the superior and ventral end of the superior meatus, from the superior and inferior recesses of the superior meatus, and from the meatus suprema I. The latter meatus persists in about 62.5 per cent of cases.

30. We should consider the adult ethmoidal region as usually presenting three conchae rather than two.

31. In the adult the posterior ethmoidal cells open into the meatus nasi superior and suprema I. The latter meatus is present in 62.5 per cent of my adult specimens, and in 75 per cent of instances this meatus has a posterior ethmoidal cell communicating with it. The superior meatus has in 100 per cent of cases a posterior ethmoid cell communicating with it at its ventral

and superior termination. The superior meatus has also in 26 per cent of instances a cell opening into the superior recess, and in 50 per cent of cases a cell opening into the inferior recess.

32. In the adult the anterior ethmoid cells open into the meatus medius. According to my series of specimens, in 100 per cent of instances anterior ethmoid cells have their ostia in the position of the suprabullar recess or furrow. The cells that open in this position vary in number from one to three, and are directed variously behind the bulla ethmoidalis. They may also be directed towards the cribriform plate or towards the frontal sinus. I find that in 13 per cent of cases there is an ostium of an anterior ethmoid cell on the medial surface of the bulla ethmoidalis, or in the position of the original bullar furrow. In 11 per cent of instances an ostium of an anterior ethmoid cell is found in the position of the infrabullar furrow. The anterior ethmoid cells that develop from the frontal furrows vary in size, number, and position. These cells may open directly into the middle meatus or into the superior and ventral end of the infundibulum ethmoidale. Some of them also frequently open into the suprabullar recess.

33. Ethmoid cells frequently extend into the body of the concha nasalis media, the agger nasi, and the processus uncinatus. These cells also at times encroach upon the cavities of the frontal and sphenoidal sinuses.

34. The ostium of the adult sinus maxillaris is frequently duplicated. This may be due to a double pouching of the primitive sinus maxillaris, or it may be developed later in a manner similar to the formation of the ostium maxillare accessorium.

35. The sinus maxillaris has, according to my series of specimens, an accessory ostium communicating with the meatus medius directly in 42.4 per cent of cases. I believe that this accessory ostium is formed by the developing sinus maxillaris, *i.e.*, the sinus maxillaris developing until the medial wall of the cavity becomes thinned out and attenuated in the position of the accessory opening, until an ostium is formed. The accessory ostium is not present in the fetus nor in the young child.

36. In the adult the sinus frontalis may communicate with the meatus nasi medius in one of the following ways: (1) The nasofrontal

duct or the sinus frontalis may open directly into the meatus medius; (2) The nasofrontal duct may be directly continuous with the infundibulum ethmoidale; (3) The nasofrontal duct may be continued down to the infundibulum ethmoidale and meet the latter structure at varying angles; (4) The sinus may have two nasofrontal ducts which in turn may have either of the above relations with the cavity of the nose; (5) Rarely the sinus frontalis communicates directly with the sinus maxillaris.

37. In some case the sinus frontalis has no true nasofrontal duct, but the sinus cavity itself extends well down into the superior and ventral portion of the meatus medius.

38. It is not common for the infundibulum ethmoidale to be *directly* continuous with the nasofrontal duct. As a rule the nasofrontal duct meets the infundibulum ethmoidale at varying angles. There is frequently a shallow groove connecting the two channels. However some specimens show that the infundibulum is directly continuous with the nasofrontal duct.

39. If we include the cases in which the infundibulum ethmoidale is *directly* continuous with the nasofrontal duct, and those in which the nasofrontal duct meets the superior and ventral end of the infundibulum ethmoidale at an angle, they will represent approximately 56 per cent of my specimens. The specimens in which the nasofrontal duct communicates directly with the meatus nasi medius will include approximately 46 per cent of my specimens. The cases in which no nasofrontal duct is present, and those in which two nasofrontal ducts present are distributed among the above specimens, since they can all be thus classified.

40. From a *practical point of view* the infundibulum ethmoidale acts as a gutter communicating between the frontal region and the maxillary sinus in 56 per cent of my cases.

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# THE THYREOID GLAND OF THE TELEOSTS

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TWENTY-ONE TEXT FIGURES AND FIVE PLATES

During the summer of 1909 at the suggestion of Dr. C. R. Stockard<sup>1</sup> I undertook the study of the distribution of thyreoid tissue within the gill region of Teleosts, especially of the trout. It seemed important to clear up certain doubtful facts in this connection, since this organ of the trout is liable to disease and at present is attracting considerable attention in cancer research. The examination of but a small number of species brought such an abundance of interesting material to light that I determined to carry out a comparative study of the anatomy and histology of the thyreoid gland in a large number of Teleosts, and to summarize our entire knowledge of the organ in this group of vertebrates. At the same time I attempted, by comparing the present results with the facts known of the thyreoid in other classes, to define more clearly the features of this organ in the entire vertebrate group.

It gives me pleasure to express my best thanks to The Wistar Institute of Anatomy and to Prof. F. R. Lillie for the use of a room in the Marine Biological Laboratory at Woods Hole, Mass. Some of the species were obtained from the New York Aquarium, for which I wish to thank the Director, Mr. C. H. Townsend.

Twenty families of Teleosts including twenty-nine species were investigated, the detailed description of which I give in the special

<sup>1</sup> My thanks are due to Professor Stockard for many suggestions during the work and for carefully revising this paper.

exceptionally occurs behind the last branchial arch. The single parts of the basibranchiale define this 'thyreoid region' dorsally, while ventrally the paired musculus sternohyoideus is spread out beneath the organ. This region is at the same time that of the ventral aorta and its branches to each of the gills.

Thus the thyreoid gland is located along the trunk through which the blood for the entire body is pumped from the heart into the respiratory organs. The narrow cleft between the bony parts of the floor of the pharynx dorsally and the muscles ventrally is completely filled with thyreoid tissue except for the space occupied by the large arterial trunks. This region, as we see from the extensive literature on the visceral skeleton and musculature in fish, in the manifold development of its bony (cartilaginous) and muscular parts shows a decided tendency to vary. It is only natural that this tendency should be found in the thyreoid gland also; since it has to accommodate itself to the configuration of the tissues just mentioned, a pronounced adaptability must be of the greatest benefit to it. The property of variation is possessed by the thyreoid gland of the Teleosts to a most striking degree and within the same species rather remarkable differences are found. Twelve weak-fish (*Cynoscion*) for instance, all differed in the extension and position of their respective thyreoids. Similar conditions were observed in other species. This variability within the species may indicate that in the thyreoid gland we have a very unstable organ, which perhaps in vertebrate phylogeny has not yet acquired its final condition. We know that in the higher classes of vertebrates there is the same variability among individuals regarding the development of their thyreoid glands. In mammalia the individual variation is very great. The lobes may have different forms, and give to the organ a paired appearance, or there may be a more or less well developed isthmus between them. Interesting comparisons have been made especially in man.<sup>2</sup> In the phylogenetically younger epithelial bodies the variation is still larger. All of these facts indicate that the gill slits and their

<sup>2</sup> Marshall ('95) examined the thyreoid glands in sixty children in which he found all possible variations.

derivates are still easily modifiable and do not yet represent a permanent condition.

The thyreoid gland of Teleosts is not a single compact organ, as we find it in the higher vertebrates, where the small parts of the gland, the follicles, are united into one complex and enclosed by a common capsule of connective tissue. Only in such a case would the term 'gland' be justified, since here numerous anatomical elements possessing the same physiological function are closely connected. The Teleosts, however, possess numerous elements, whose totality from a physiological standpoint one must regard as a thyreoid gland, while anatomically we are unable sharply to define the organ in question in this group of vertebrates. In most cases we can speak of thyreoid follicles only, or groups of follicles, in pointing out the distribution of the organ. Thus in plates I and II, not the thyreoid glands of the respective species, but the regions of distribution of the thyreoid follicles themselves are figured. On plate III, however, which indicates the variation in the location of the gland within the species, real parts of the thyreoid are indicated, so far as they were macroscopically visible.

In the more closely defined region we may find thyreoid tissue in all parts. It is usually of a brownish yellow color. The follicles are generally most densely located in the neighborhood of the ventral aorta or of the branchial arteries. Those places, particularly, are favored where the branchial arches arise from the aortic stem. Follicles are most abundant at the origin of the second gill arteries, that of the first being next, and finally the roots of the last branches have fewest thyreoid follicles about them. A more or less dense accumulation is always found along the stem of the ventral aorta, which may be completely surrounded by thyreoid tissue. In other cases dense accumulations of follicles are located either dorsally or ventrally to the aorta.

The glandular tissue is usually distributed so that it is more densely accumulated in the central region, while towards the periphery a more and more pronounced dissolution and scattering of the follicles takes place. These conditions cannot, however, be strictly generalized, as we find cases in which, even in the most central portions, the follicles are not more closely arranged than

in the peripheral. In some instances, as in *Cynoscion*, rather well developed central portions are found which can be recognized by the naked eye. But even in these cases numerous follicles lie well separated from the main body. Thus we have all transitional stages from a perfect dispersion of the follicles to a rather compact union of them. It is possible that further investigations may show cases in which the organ is still more compact than in those thus far examined, and so present a structure similar to that in higher vertebrates. Judging from my observations, however, this does not seem probable, and at present I am inclined to regard the conditions found in *Sarda* (*vide* Special Part) as the limit of compactness in the series.

The cephalad and caudad extensions of the thyroid gland vary very much. In general, it might be said that a spreading out toward the tip of the tongue takes place in all cases, while towards the heart the distribution is not so uniform. Far cephalad of the first aortic bifurcation we find single follicles scattered below the hyoid bones. The caudal limit of the thyroid gland usually lies between the second and third aortic branches, or at the third. Rarely does it go beyond this point, and if so, with a few exceptions, only scattered follicles are found in the posterior region. Thus we find an accumulation of the glandular elements around the anterior part of the ventral aorta, with follicles scattered towards the head and the heart.

The organ decreases in mass in an anterior-posterior direction. In one instance, *Siphostoma*, just the reverse is the case.

The embryonic center from which the thyroid starts to grow lies between the first and second gill branches, this place in the adult animal is near the aortic bifurcation, and in many cases we find the main part of the organ in this region; while in other cases, *Cynoscion* and *Tautogolabrus*, it is exclusively there. From the region of the aortic bifurcation the thyroid elements travel so as to occupy the different positions which are described in the special part of this paper. The migration of thyroid follicles occurs more particularly in the direction of the heart, although a cephalad migration is decidedly pronounced. The development of the hyoid bones obstructs the anterior spreading

of the follicles to some extent. The follicles apparently tend to go around the basihyale and along its sides towards the tip of the tongue. In the dogfish and shark where the hyoid region offers a comparatively free space we find it occupied by the compact thyreoid, which is pushed slightly forward of the aortic bifurcation (Ferguson). In these animals also the thyreoid is originally placed in the bifurcation of the truncus; later, according to W. Müller, the anlage moves forward and becomes encapsuled. In *Raja*, on the other hand, it remains in the bifurcation.

The dorso-ventral, in combination with the lateral, extension of the thyreoid follicles seems to be more dependent upon the configuration of the pharyngeal floor than does the cephalo-caudad extension. In fishes, in which the isthmus region is deep and narrow, as in *Brevoortia*, we find, as might be expected, the dorso-ventral distribution of follicles far surpassing the lateral. While in other species, for instance, *Tautoga*, in which the floor of the pharynx is very broad, the lateral extension is the important one. In general it may be said that the lateral outweighs the dorso-ventral distribution of follicles.

Dorsally the follicles are usually found between the ventral aorta and the copulae of the gill arches. In cases where those skeletal parts come close to the vessel the follicles are forced away laterally, and sometimes intrude into the spaces between the copulae and hypobranchialia. When the parts of the basibranchiale lie well separated the follicles extend up between the copulae and come to lie close to the mucous membrane of the pharyngeal floor.

The main mass of the organ lies almost exclusively above, or dorsal, to the ventral aorta. This is opposed to Maurer's statement of the case. Below the aorta is usually found the smaller part of the gland and the follicles are also more loosely scattered. The development of the thyreoid gland above the aorta should be expected since there is usually much more open space between the aorta and the gill arches than is found below the aorta where the muscles lie close to the vessels. The thyreoid elements endeavor to intrude below the aorta as much as possible, and when this vessel, in the region of the third branchial arteries, sinks deeper



into the musculus sternohyoideus, the follicles follow in the course and are thus distributed far ventrally within the muscle. The *number* of follicles along the sides of the aorta is always less than either above or below it. This, by no means contradicts the statement made above that the lateral *extension* of follicles outweighs the dorso-ventral one. Only in the genus *Fundulus*, especially in *majalis*, less so in *heteroclitus*, where a transverse inter-branchial muscle pushes the vessels away from the skeletal parts, is the dorsal extension small, or sometimes lacking entirely. Here the follicles extend directly away from the aorta towards the bases of the gills.

Along the aortic stem between the bases of the gill arteries the lateral extension is somewhat limited and reaches its height along the branchial arteries. The vessels seem to serve as bases along which the follicles migrate. The free space about the gill arteries becomes narrower and narrower as the gill arches are approached and therefore the number and size of the follicles decrease towards these points until there is no more room for extension. When, however, there exists an especially open passage along the vessels the follicles may even extend into the gill arches, to a considerable distance beyond the point of their origin. Such cases are common in trout (text fig. 7D).

The peculiar distribution of the thyroid elements forces us to regard the organ in bony fishes as unpaired, a view also supported by embryology. This statement should be especially emphasized, as in Wiedersheim's *Comparative Anatomy*, 1907, the author still speaks of the thyroid gland in the Teleosts as a paired organ, although Maurer in 1886 (p. 134) criticizes this statement in an earlier edition. On another page, (p. 140) Maurer himself claims that the main bulk of the organ at a certain stage is not paired, while later single portions of the gland lying in the median line, as well as on both sides of the truncus arteriosus, take a paired arrangement. This is certainly incorrect, since the follicle groups on the sides of the aorta are not only unpaired but are also not bilaterally arranged.

The relationship between the thyroid gland and the stem of the ventral aorta is purely anatomical and without any physiological

importance. The thyroid does not receive its blood supply from this group of vessels, since they carry only venous blood, and the arterial blood which nourishes the gland comes from a special thyroid artery. In *Petromyzon*, however, Cori claims that the *arteria thyreoidea* arises from the *truncus arteriosus*; this is probably an error, as he also finds the ventral carotid connected with the *truncus*. The thyroid artery, as Silvester demonstrated in *Lopholatilus* and twenty other species of Teleosts by his perfected method of injection, arises as a dorsal branch from the united right and left fourth commissural arteries. The latter vessels originate from the second efferent branchial arteries and unite in the median line below the thyroid and the aorta as the hypobranchial artery. Shortly after the union of the fourth commissural arteries the thyroid artery branches off from the dorsal side and immediately enters the gland in its posterior region. Whether the widely scattered follicles all receive their capillaries from this one vessel cannot at present be stated, though it would seem very doubtful especially in the case of the more anteriorly isolated follicles.

In Selachians, where the thyroid is pushed far forward, the *arteria thyreo-spiracularis* (Dohrn) originates in the first aortic arch from the *arteriae efferentes* of the hyoid gill. In Teleosts, also, the first aortic arch breaks up into a capillary network. Dohrn, therefore, speaks of an *arteria thyreo-spiracularis*. Perhaps it is from this vessel that the most cephalad parts of the thyroid gland receive their blood supply. The artery pointed out by Silvester seems, however, to supply the bulk of the organ, and the term *arteria thyreoidea* as applied to it is apparently justified.

It is of interest to recall Simon's statement, which was also supported by others, that the thyroid gland is placed in the blood system so as to regulate the supply of blood to the brain. This, in a way, was a foreshadowing of our present views that the physiological action of the thyroid gland exerts an important influence on the central nervous system.

The venous blood from the thyroid gland passes into the thyroid vein, a vessel, which also collects the veins from the muscula-

ture below the aorta and carries the blood directly into the sinus venosus.

Little is known about the relation of the thyroid gland to the lymph system. This is largely due to the fact that in the fishes the lymph vessels are in a much closer connection with the venous system than in the higher vertebrates. It is almost impossible to distinguish between veins and lymphatics by the injection method.

In many species large cavities lie around the aorta, two dorsal ones being constant in trout. These are extraordinarily large and lined with endothelium and although they often contain blood corpuscles there is little doubt that they are lymph sinuses. The corpuscles probably come in from the venous system, or possibly by traumatic haemorrhages. A further fact in favor of their being lymph sinuses is that no descriptions of large veins in this region has come from the numerous injections of the circulatory system of Teleosts. In some species there is only one large 'lymph sinus' which surrounds the aorta dorsally and laterally.

#### DEVELOPMENT OF THE THYROID

Only one contribution deals with the embryology of the thyroid in Teleosts, this is by Maurer ('86) who traces its development in the brook trout. The gland arises in much the same manner as it does in the other classes of vertebrates.

The thyroid develops very early in the Teleosts, after the first gill slit has broken through,<sup>3</sup> as an unpaired evagination of the stratified epithelium on the ventral side of the pharynx between the first and second gill pockets. It is thus placed in the curve of the S-like tubular heart before the gill arteries have developed, with the exception of that to the hyoid arch. The vesicular thyroid anlage very soon separates itself from the pharynx and enlarges by budding. The organ lies close to the tubular heart, but only remains for a short time near the place of its origin. With the development

<sup>3</sup> In Hertwig's Handbuch d. Entwicklungslehre II, 1, 1906, Maurer states, however, that the anlage of the thyroid appears in all Gnathostoma before the breaking through of the first gill slits.

and shifting of the heart and aorta as well as by its own growth the thyreoid gland comes to lie far from its original position. The absence of a capsule of connective tissue similar to that in higher vertebrates admits the loosening and separation of the thyreoid follicles in the bony-fish.<sup>4</sup>

The Teleosts show a condition of the thyreoid gland somewhat similar to that in *Myxine glutinosa*, as W. Müller, Cole, Schaeffer and Maurer state. The follicles in *Myxine*, partly isolated, partly in groups, are found between the pharynx and the truncus arteriosus throughout the gill region. In the Teleosts, however, the gland also extends below the truncus. In the skate Baber observes "a single body and a few detached vesicles"; in the Amphibians separated particles have also been described. Yet in both of these groups the thyreoid possesses a capsule, which sends septa into the inner portions, as W. Müller has shown for *Acanthias* and *Raja*. Maurer finds a delicate connective tissue capsule in the Urodela. These observations on Selachii and Amphibia, however, are exceptional and the small detached particles can only be looked upon as 'aberrant thyreoids' the main thyreoid in all cases being a sharply defined body. Maurer observed in the Urodela that a breaking up of the thyreoid into smaller parts occasionally occurred. These accessory thyreoid glands were parts of the former isthmus which, after the anlage had divided, persisted and remained in their original position, while the true halves moved in a postero-ventral direction. In the Ophidia the organ is compact and encapsuled; but in the Saurii, according to W. Müller, the interstitial tissue increases so much through the accumulation of fat, that the glandular tissue proper is broken up into irregular groups which are sometimes completely disconnected. We have here a dissolution within the capsule suggesting that the connective tissue capsule is the only factor in other vertebrates which prevents the thyreoid elements from becoming scattered about as they are in Myxinoids and Teleosts.

<sup>4</sup> The elements of the Teleost pancreas are similarly scattered in the mesenterium.

During development of the Teleosts some follicles cling to the wall of the aorta and are in later life found along it. Usually the follicles become arranged into several distinct groups, forming different centers of growth, as is shown by *Cynoscion* in plate III. With the branching off of the gill arteries from the aorta thyreoid material is carried out laterally towards the gill's and spreads in this region. This accounts for the larger lateral extension of thyreoid follicles along the gill vessels rather than in intermediate regions. The larger vessels form a substratum upon which the follicles migrate as do also the smaller vessels and especially the lymphatic vessels. The larger vessels are means for the antero-posterior dispersion while the smaller ones allow the migration of follicles from the denser central thyreoid portions towards the periphery. Even the most peripheral follicles are usually found near blood capillaries although they do not necessarily come in close contact with them. The way in which these isolated follicles function is not clear. They certainly seem normal and contain colloid.

The growth of the connective tissue and fat in which the follicles are imbedded favors their dispersion from the central portions; thus a combination of influences are at work to widen the thyreoid region as much as possible. W. Müller regards the immense development of the 'interstitial' tissue as alone responsible for the dissolution of the thyreoid into isolated groups. He no doubt refers to fat and connective tissue, as we shall see below that the term 'interstitial' is not properly used in this case. Although the growth of these tissues may be an important factor I do not regard it as primary, since in the first place, even in young individuals, the follicles are found isolated, and secondly, the breaking apart of a formerly compact organ by excessive growth of connective tissue would certainly not account for the carrying of the follicles into the muscles and gills.

The follicles actually seem to overcome the obstruction offered by other tissues in their course and may even penetrate into them. In trout and *Micropogon* the thyreoid follicles are at times imbedded in the muscle tissue, into which they creep between the connective tissue lamellae or along the blood vessels. Real activ-

ity on the part of the follicles is most unlikely, and the probability is that they are simply passively pushed or pulled as circumstances may have it. The forces in development unite to make it possible for the thyroid gland to spread, and so form a greater amount of functional tissue than could be contained in a compact organ situated in the narrow space between the basihyale and the ventral musculature.

Little is known of the manner in which the thyroid gland grows and forms new follicles, and contradictory statements are also found in the literature regarding the primary anlage of the organ. It is scarcely conceivable that a vesicular anlage should exist in all fishes except *Ceratodus* in which Greil observed a solid one. Before the solid outpushing in *Ceratodus* separates from the pharyngeal wall it is said to become vesicular, a process exactly the contrary to the usual one.

Amphibians are believed to have a solid bud-like thyroid anlage. Maurer states that two days after its evagination the thyroid is solid in the *Anura*, and W. Müller observed a solid anlage in *Rana temporaria* and *platyrrhinus*, in which the first lumen appeared in 25 mm. larvae, after the gland had divided into two halves. In the *Urodela* Maurer records a solid epithelial bud, Livini finds the same in *Salamandrina perspicillata* and Muthmann in *Triton alpestris*. Platt claims that Maurer's description does not apply in all the *Urodela*, as is shown by the condition in *Necturus*.

The reptiles, birds and mammals are said by the majority of observers to show a vesicular thyroid anlage, which changes into a compact organ from which follicles later originate. Kölliker, however, observed in the rabbit a thickening in the ventral wall of the pharynx, from which a wart-like solid process was cut off. Born also records the same for the pig. (Both authors quoted from Streckeisen).

This point is of importance in phylogenetic interpretations since our present views regarding the ancestry of the thyroid gland are mainly based on a similar evagination, that for the endostyle, found in the *Tunicates* and *Amphioxus*.

Maurer finds in the trout a primarily globular vesicle stretching in an antero-posterior direction, and on the 41st day of development lying ventral to the stem of the aorta. If these statements apply to all Teleosts, the thyreoid must first originate dorsal to the aorta and early migrate ventrally and later return to a position dorsal to the vessel, since it usually occurs there in the adult. The condition in the Teleosts is similar to that in the Myxinoids, where Stockard describes the origin of the thyreoid as a median down-pushing from the ventral floor of the pharynx throughout the entire gill area, and consisting, in newly hatched *Bdellostoma*, of diffusely scattered alveoli below the pharynx and above the median branchial artery (ventral aorta).

In the trout, where development is rather slow, Maurer observes that 35 days after fertilization, when the embryo is about 6 mm. long the first thyreoid vesicle begins to pinch away from the pharynx. While originally the evagination, visible on the 28th day, possesses a stratified epithelium, it has on the 35th day a single layer of cuboidal cells. Three weeks later the whole stem of the aorta is surrounded by follicles. I find in rainbow-trout, one month old, or only 30 days older than those mentioned by Maurer, that the majority of follicles, and the larger ones, lie above the aorta.

Maurer also observes that in the brook-trout shortly after the first follicles have appeared the organ grows so rapidly that for a considerable period it surrounds the aorta as a compact mass. "In very late embryos, the growth of the thyreoid does not keep pace with that of the artery; thus the gland breaks away from the aorta and separates into a number of irregular clusters of different sizes lying either laterally partly paired or dorsal or ventral to the aorta, always, however, in its immediate neighborhood." He records the main mass of the gland in trout of even 25 cm. as being compact and situated ventrally between the second and third branchial arteries, and it is only in animals of 30-40 cm. that the thyreoid breaks up into the clusters of follicles characteristic of the adult. Maurer describes the same conditions in a number of other species, of which only the eel was at my disposal. The age of the eel I examined was unknown, though



according to Maurer it must have been rather old, yet it measured only 30 cm. long.

Maurer's observations do not accord with the conditions I find in rainbow-trout four weeks old, nor in 25–30 cm. brook trout. As before stated, there is no paired arrangement of the thyreoid clusters, and the follicles are also in many cases distantly removed from the stem of the aorta. Other differences may be either due to specific or individual variations. Maurer's statements would indicate that the thyreoid gland tends to preserve its original unity, being finally broken up by force. My observation, however, seems to show the contrary, at least in *Salmo irideus* and *fontinalis*. In individuals one year old the follicles are more densely packed than in those one month old, although the intervening spaces have grown larger. The follicles also have become more numerous. This seems to warrant the supposition, that the thyreoid elements are disassociated at an early stage and subsequently multiply.

The multiplication of the follicles is described by Maurer as being very simple. While the epithelial cells are increasing in number after the forty-first day (in trout) solid buds appear on the primary vesicle, which very soon form central cavities and then pinch away. We do not know whether a similar process is maintained in later life, follicles coming from follicles, or whether new follicles are derived only from primary epithelial cells multiplying and forming a lumen. The latter supposition would more readily explain the scattering of thyreoid elements, germ elements I might say, to distant regions. L. Müller believes the new follicles to originate from old ones by buds from the epithelium which are subsequently pinched off. Baber contributes an interesting observation in the conger eel where in the wall of large follicles small ones sometimes lie imbedded so deeply that the epithelium between them is flattened out. Baber thinks that at times the wall breaks through and the two lumina are united. In other cases, however, the small imbedded follicles grow out and become independent.

The epithelial tubes found in the thyreoid of higher vertebrates as transitory growth stages are absent in the Teleosts. In the



Urodela a solid cylinder of epithelial cells from which the follicles pinch away, exists for from two to four weeks. These cylinders are observed in sheep and pig embryos up to the 20 cm. embryos and in man up to the 24 cm. embryo. The follicles in fish are formed comparatively earlier, and perhaps the gland functions earlier.

Thus a rapid multiplication of follicles occurs in the Teleosts without the formation of cell cylinders. This is an exception to W. Müller's claim that the thyreoid gland in all vertebrates passes through three stages: (1) a severing of the anlage from the pharynx; (2) formation of a network of tubes of glandular epithelium; and (3) the formation of follicles from these tubes. In the Teleosts and also in the Myxinoids, as Stockard has shown for *Bdellotoma*, the second stage seems to be suppressed or absent.

The first appearance of colloid in the thyreoid gland is generally thought to occur early in lower vertebrates but very late in the higher ones, towards the end of fetal life or often not until extra-uterine life.

Maurer reports colloid in the trout thyreoid on the forty-first day of embryonic development. How far this early appearance of colloid is connected with the function of the organ is unknown. From a comparative physiological standpoint it would seem that in the lower forms the thyreoid might function much earlier than in the Placentalia, where in intra-uterine life the gland of the mother might supply the needs of the developing embryo.<sup>5</sup>

#### HISTOLOGY OF THE GLAND

The histological structure of the thyreoid gland in Teleosts has been little studied. The meagre observations made by Baber in 1881, describing some features of the thyreoid in the conger eel were the first reported. Maurer later ('86) mentions a few points regarding the histology of the thyreoid in the trout and carp.

The microscopic appearance of the gland varies as much as does its anatomical structure. In sections from some specimens the

<sup>5</sup> In young mammalian embryos Peremeschko found no colloid, in older embryos it occasionally existed, while in young animals colloid was present in the majority of follicles and in old ones in all of them.

follicles are closely arranged and so densely packed that apparently only lymph spaces exist between them, in others we find the follicles more loosely connected and suspended in the connective tissue; while again in other specimens they lie so far apart that they can scarcely be thought of as belonging to one organ. The histological appearances also differ very much within the individual, depending upon the region from which the section is taken.

When the arrangement is such that the thyroid may be dissected out and then sectioned, the follicles are found to be rather densely packed (pl. V, fig. 17). By this method, however, we are unable to get a correct idea of the extension of the thyroid and the arrangement of its follicles, since it is only possible to remove the somewhat denser masses around the stem of the aorta, usually near the base of the second aortic arch, and all the particles in front and behind this region still remain. Properly to study the general distribution of the thyroid follicles serial sections through the entire gill region are absolutely essential.

The spaces between the muscles, branchial arteries and gill arches are filled by wide-meshed connective and fatty tissue. In these tissues the follicles are suspended. The connective tissue is, therefore, not so directly a part of the thyroid organ in these fish as it is in the encapsuled organ of mammals. The primary object of this tissue is to form a connection between the muscles and bones without regard to whether there may be thyroid tissue in the region or not. True interstitial tissue, as such, is not found in this diffusely scattered thyroid organ. Of course, the tissue in which the follicles lie imbedded performs the same function as does the capsule in higher vertebrates: in both cases it serves to support the follicles. In glands, where many follicles are accumulated in one mass, as in *Cynoscion*, or in the central portions of some others, for instance the trout, the supporting tissue may be regarded as part of those masses, but not as part of the entire thyroid gland; here also the formation of connective tissue is the primary process, and the suspending of the follicles only a secondary one.

The supporting tissue is simple except in two species, *Salvelinus* and *Sarda* (pl. V, fig. 21) where smooth muscle fibres are freely

suspended in the connective tissue. These muscle fibres are found especially below the aorta, where they approach the follicles and at times surround them. This is accomplished by the fibrillae of a bundle loosening up a little, then enclosing a row of follicles and finally uniting again.<sup>6</sup>

Regarding the number, size and form of the follicles, all variations exist which have been demonstrated by comparative investigations in the other classes of vertebrates. The size of the follicles is, in general, in reverse proportion to their number. The size, however, is not of great importance, since the chief factor in the activity of the gland is the epithelial surface; this will be the larger, the greater the number of small follicles contained in a given region. Biometric calculations would be interesting in this direction as experiments have shown that the functional value of the thyreoid gland varies with the individual. Glands are found in which the size of the follicles is uniform; in such cases the follicles are usually large. As a rule, however, the follicles are of various sizes as would be expected in view of the process of formation of new follicles. In many cases I have observed that a few (three or four) follicles are unusually large.

The follicles lying in the central parts are generally larger than those towards the periphery. This seems quite natural in view of the mode of extension of the gland. In only one case, Sarda, do the central portions consist of nests of numerous small follicles while larger follicles lie peripherally (text fig. 12). This condition resembles somewhat that in birds (Baber), and mammals (Anderson, Forsyth) and if it be due to the fact that in Sarda the gland is almost as compact (of course without a capsule) as in the higher vertebrates (with capsule) then we must suppose that in such a case new follicles are formed in the centre and are pressed out towards the periphery, while in the breaking up of the gland minute parts are continually carried towards the periphery and there form new follicles. In the first case the peripheral follicles would be the oldest and in the second the youngest ones.

<sup>6</sup> Streiff finds muscles between the glandular tissue in the thyreoid of the cat, Zielinska in a young dog, Wölffler in a child (cit. from L. Müller), L. Müller in an adult woman. The muscle must have migrated into the gland during the first half of the embryonic period, before the capsule was formed.

The form of the follicles is also variable, most typical perhaps are the globular or elliptical and tubular types. The smaller follicles are nearly always circular in section (pl. V, figs. 10–12), especially when they are free. The shape of the more closely packed follicles is influenced by pressure, and may be flat, indented, or irregular in outline. When the follicles lie next to the cartilages or muscles they are usually oblong-oval, with the longer side towards the tissue. Single follicles lying in the supporting tissue, if large, are rarely perfectly circular, but have irregular outlines due to pressure from the fibres of the substratum. The shape of these follicles indicates the existence of actual pulling forces in the supporting tissue.

Not only small irregularities are found in the surface of the follicles, but also deep invaginations of the epithelium as well as long evaginations. The follicle may consist of a central body with sprouts or branches of cylindrical and globular shapes (pl. V, figs. 15, 16). How far these irregularities in form are connected with the cutting off of smaller follicles from larger ones could not be determined. Anderson doubts the multiplication of follicles by such a process.

It is now generally accepted that no communication from follicle to follicle exists; the follicles are closed on all sides and perfectly separated from each other. Sometimes, however, as many as five follicles are observed in a section, apparently perfectly separated, but on tracing through the series of sections they all unite into one follicle (pl. V, fig. 16). This is due to evaginations from the follicular wall somewhat like the fingers of a glove, which when cut across, give the appearance of several independent follicles, while in reality there is only one lumen. In *Anguilla chrysopa*, however, there really seemed to be a communicating duct between two follicles; the lumen of the tube was much narrower than that of the follicles and the epithelial cells of it were much higher (pl. V, fig. 15). This closely resembles a 'Schaltstück' as seen in other glands. This was not due to a waist-like constriction of the epithelium, but to a far reaching evagination from one follicle with a globular swelling on the free end representing a second follicle. There was no colloidal substance in the 'intercalary' duct.

Branched follicles are particularly abundant in some species. In *Muraenoides* all follicles seem to branch. Baber states that in young animals the follicles are much more ramified than in older ones; he, therefore, regards this branching as the method of follicle multiplication. Anderson, on the other hand, holds the 'melting' of the epithelium (a process about which I shall speak later) at the point where two follicles meet responsible for the communication between several lumina; this of course is an opposite process from that of budding. Anderson, therefore, believes that in old animals there are more irregular follicles than in the young. It seems to me that the ramification of the follicles does not depend so largely upon age, but rather on the species.

The follicular epithelium varies but little with the species, perhaps the number of cells may differ in follicles of the same capacity. The epithelium is of the form usually found in the thyroid glands of higher vertebrates. All transitions exist from a pavement epithelium of very low broad cells, through cuboidal cells as high as broad, to very high and narrow cylindrical cells (pl. V, fig. 10).

The form of the epithelium is probably connected with the age of the specimen, as it undoubtedly flattens with increasing age. (In very old human subjects only perfectly flat cells have been found.) Age, however, can scarcely be the only factor, as in some species different forms of epithelium appear at the same time. This may be due to the different ages of the follicles, though it cannot be regarded as an absolute rule that the older follicles have a lower epithelium than the younger. Hürthle definitely states that these two factors are independent of one another. Langendorff points out that the follicles increase in size, not by a flattening out of the epithelium, but by multiplication of cells. I should say that both processes may be simultaneously involved since we often find large follicles with high epithelium, yet karyokinesis is rarely observed in the epithelial cells. The latter fact led Stockard to suppose that amitotic cell division might occur in the growing thyroid tissue of *Bdellostoma*.

The different types of epithelium might be accounted for in still another way by supposing the follicles to be in different

stages of activity. Here again we meet with difficulties since the same follicle sometimes shows high cylindrical epithelium on one side and a flattened epithelium on the other (pl. V, fig. 11). Hürthle considers the flattening and stretching of the cells to be the final stage in the process of colloid formation. He finds this type of cells not sporadically, but always in the larger groups of follicles. The low epithelial cells are still alive and according to Hürthle may again transform into high ones. Biondi claims that when the follicle has reached a certain size, the epithelium partially flattens and vanishes, thus establishing a communication between the follicular lumen and the lymph spaces and allowing the colloidal material to be poured into the lymph system. The emptied follicle is said to collapse and from its cell mass a new follicle originates. Anderson, also, thinks that by a 'melting' of the epithelium a connection is formed between the lymph space and follicle, but the individual follicle is not destroyed.

In the conger eel Baber finds oval cells between the cylindrical ones and attributes to them the formation of new follicles, an idea which I think is incorrect. The two classes of cells could not be found in the common eel. Baber also finds in the conger eel 'club-shaped' cells between the epithelial cells. They are much narrower than other cells and possess elongated nuclei. Their free ends project above the general surface and are expanded 'fan-like;' the bases may also show a similar condition. Baber regards them as branched cells, often existing in pairs, and forming stomata which play an important part in absorption and secretion. If this be true such cells should exist in all glands. I was unable to find these and think perhaps they may have been consequences of his alcohol preservation.

The form of the nucleus changes with the form of the epithelial cell. It is usually circular or somewhat oval in cross section. When the cell is either cylindrical or flattened, the nucleus becomes more and more elliptical in shape, with its long axis parallel to that of the cell. Thus in the first case the long axis of the nucleus is vertical to the free surface of the cell and in the second parallel with it. When the nuclei are oblong in spite of the cells being broad and cubical (for instance in some follicles of *Muraneoides*)

they always present the long side to the free cell surface. Frequently narrow cells with oblong nuclei are seen between the cuboidal cells.

In trout degenerating epithelial cells of small size were observed with compact nuclei, deeply staining or pyknotic.

The nucleus usually lies at the base of the cell (pl. V, fig. 11) but may sometimes, especially in an epithelium with many cells, move a little towards the lumen (pl. V, fig. 10). Nuclei may lie at different altitudes, in an alternating fashion. One or two nucleoli are visible.

The shapes of the nuclei usually give no indication of the state of activity of the cell as Anderson has claimed. Even pyknotic nuclei usually have regular outlines. An exception to this is seen in the trout where often, in some varieties almost exclusively, epithelial cells show nuclei of very irregular shapes, as indicated in pl. V, fig. 10. The nuclei are elongated with more or less bent corners—horse-shoe shape. These were generally found in lower cells; they may have been degenerating, since they did not stain as deeply as the normal ones in other parts of the gland, when such were present. It seems, however, scarcely conceivable that the epithelium of the entire gland should degenerate, unless from some pathological condition. (These animals were all reared in the N. Y. Aquarium.)

The cytoplasm of the cell appears granular and sometimes stains slightly darker in the basal region. There is no cuticle lining the lumen, but the refraction of light in this region has misled some authors. The base of the cells is usually rather smooth, though in cases where vessels come into close contact with the follicle the straight basal line becomes somewhat interrupted through the influence of the surrounding structures. In Brevoortia the epithelial cells are nearly all drawn out as if they possess projections. Those of one follicle approach very closely those of others and it seems almost as if a connection between the follicles were established (pl. IV, fig. 1, 2). Other somewhat broader cells possess pedicel-like bases which are sometimes branched, giving the impression that the cells are sending out pseudopodia. The processes disappear in the interfollicular tissue in close contact



with the blood and lymph capillaries. The process cells are limited in number and lie close together. Their cell body is swollen with foamy cytoplasm containing several deeply stained highly refractive granules. Perhaps these cells are in a state of degeneration, probably colloidization, although their plasma does not show any acidophilia (pl. IV, fig. 5, E). Peremeschko observes somewhat comparable features in the thyroid glands of birds and mammals, especially in that of the rabbit. Some of the epithelial cells possess at their basal end from one to ten small projections, and thus resemble the tassel cells Pflüger has described in salivary glands, except that in the thyroids the processes are shorter. In some cases Peremeschko found such cells in fresh material and could isolate these follicles, which appear to be surrounded by a fringe. Pflüger regarded the cell processes as nervous, but Peremeschko correctly believes them to come from the cytoplasm of the epithelial cells.

The function of the follicles can be much more easily studied in other groups of vertebrates than the teleosts. In dissecting out the follicles, as far as they are macroscopically visible, and fixing them, it is almost impossible to avoid destroying the finer structures. Hemorrhages are almost unavoidable in cutting open the gill region. On the other hand, in fixing the entire floor of the pharynx the fixation fluid does not penetrate sufficiently fast to preserve the finest details, and the general structures are unfavorably influenced by the decalcification process. Microchemically, therefore, little can be done and I limit myself to what could be determined from studies of general structures.

Hürthle's colloid cells were seldom seen in the thousands of follicles observed. Whether they are not generally formed, or whether they appear and are emptied in so short a time as to be rarely preserved I am unable to say. In *Clupea* (pl. V, fig. 20, Coz.) they were limited to four or five neighboring follicles and in these all of the epithelial cells were so swollen that in some cases they met in the center of the follicle, obliterating the lumen. The nuclei were compact and deeply staining and occurred directly under the free surface of the cell. The cytoplasm was homogeneous, highly eosinophile, and sharply distinguished from that of other



epithelial cells, and thus the colloid forming zone was well defined. This agrees with Hürthle's account, which states that the colloid cells always appear at the same time in a large portion of the wall of a follicle or in several neighboring follicles. The size of the follicles has nothing to do with their appearance. In one case, *Siphostoma*, the epithelium of all follicles consisted of cuboidal swollen cells, the nuclei of which were near the cell center or towards the lumen and the cytoplasm was highly acidophile, (pl. IV, fig. 8).

The normal contents of the follicles is the colloid. It is found in all thyroids and usually all the follicles contain it; only in a few cases were the majority of them empty.

In spite of the various ideas expressed in the literature regarding the surface irregularities of the colloid there is little doubt that they are caused by shrinkage in fixation. In the majority of follicles the surface of the colloid was perfectly smooth, in some a little retracted from the epithelium, but in others completely filling the lumen. In some follicles the colloid showed surface indentations. These differences can scarcely be connected with the age of the organ, as they were observed in different stages. In all the young trout, however, the colloid filled the follicles completely. One possibility is that the content of the follicles does not always possess the same chemical composition, and is influenced by the same fixation fluid in different ways.

The view has been held that the true secretion of the cells is hyaline and that it appears in the form of small droplets which are set free on the surface of the cells. This process is thought by some to be responsible for the irregular surface of the colloid. Two kinds of surface irregularities must be distinguished, first, the large ones which do not correspond with depressions of the epithelial cells. These are without doubt due to the fixation. The connecting threads of colloid between the central portion and the epithelium seem to run between the cells and to take hold there. Tangential sections through the follicle wall, cutting the epithelium just under the free surface, show that the cells do not always lie closely placed in their upper portions and a

network of colloidal threads is shown between them. In higher vertebrates these large surface irregularities in the colloid seem more common than in fish. The second, smaller irregularities might resemble secreted droplets. They give to the surface of the colloid, especially from a top view, the appearance of being beset with oil drops. In some places there are merely slight depressions in the free margin, some distance apart, while in others the whole surface is corrugated, but these irregularities do not appear in all of the follicles. Whether they are really physiological products of the cells is not determined. The irregularities may be more easily explained on the theory that where the free ends of the cells do not come in close contact, the colloid which fills the follicles is pressed into the intercellular spaces and surrounds the top of the cells like a cap. In shrinkage from fixation the caps would be pulled from the cells, leaving on the surface of the colloid the impressions. Anderson regards these 'droplets' as well as the numerous vacuoles, which he finds within the colloid even of living glands, as "cavities lined with a hyaline membrane and containing the 'chromophobe' secretion, a part of the secretory activity of the gland." Langendorff and others more correctly regard them as artefacts, having no physiological significance. Vacuoles within the colloidal substance are seldom seen, (pl. V, fig. 13, V).

The colloidal material seems to become denser with age, as far as this can be determined by its staining capacity. In young trout it is rather pink so that it can scarcely be distinguished from the blood serum in the vessels. Both structures show the same microscopic appearance. In older trout, however, the colloid stains very deeply with acid dyes. These observations agree with those of Schmid on dogs of different ages. Anderson, Boéchat, Peremeschko and others also state that the number of follicles containing a slightly staining finely granular colloid diminishes with age, being small in old individuals. I failed to find some follicles distinguished by a greater affinity for the stain than others, as was claimed by Hürthle, but did find that sometimes within the same follicle the colloid stained differently in different places.

The structure of the colloid varies with the species and is the same through all the follicles. A perfectly homogeneous colloid exists in cases, in others it is granular, and finally in some fish it is of a lumpy consistency. The consistency of the colloid also varies with age, in old animals being rather cloudy in appearance and evidently very brittle after fixation. Occasionally the outer portion of the colloidal mass stains a trifle lighter which is the only indication of a concentric structure. This alone, however does not argue for the view that the colloid is a by-product of the active thyreoid, which collects and remains in the follicle. Langendorff first presented such an idea which of course called forth great opposition.

Blood corpuscles are occasionally found in the Teleost thyreoid and sometimes completely fill the lumen of the follicles or may be scattered or bunched together. Blood is also occasionally found in the human follicles. Baber was no doubt mistaken when he spoke of a real flow of blood into the follicles, as such does not occur. How the corpuscles enter the follicles is not known, though it is probable that somewhere, by pressure or tension, the delicate wall of a capillary lying next to the epithelium is ruptured and the corpuscles find their way into the follicular lumen through an injured wall. Hürthle believes the 'melting' of the epithelium responsible, when it occurs at a place where capillaries lie deeply imbedded. It is evident that whenever blood corpuscles do enter the lumen they are destroyed, and they may be seen in all stages of disintegration until finally pyknotic shadows of nuclei alone exist with no indications of their cell bodies. The scattered corpuscles lie within the colloid, which must therefore, be rather liquid. The content of the follicles has a haemolytic property without being itself of haematogenic (Baber) origin.

Cells from the follicular epithelium also form a part of the follicle content. These are pushed off either singly or several together into the lumen and there destroyed; they also lie within the colloid. Two kinds of cells are distinguished, those with a small body and dense cytoplasm, resembling somewhat epithelial cells of the ordinary type and those with a swollen body, and

clear cytoplasm (pl. V, fig. 13, Coz), which may resemble Hürthle's colloid cells. The two types are probably different stages in the same process. At times the cell body is broken up into pieces before being transformed into colloid. The nucleus is always destroyed last.

A part of the colloid is therefore formed by degenerating epithelial cells which are either destroyed in their primary position or after being pushed into the lumen. Anderson believes that this is invariably the fate of the cells after several periods of secretion. Hürthle, also, noticed this 'melting' of the epithelium and was able to trace the complete disintegration of the cytoplasm, though the fate of the nuclei remained doubtful. They, too, are unquestionably destroyed within the colloid, and as a matter of fact I could observe cell nuclei, such as those of the red blood corpuscles, in all stages of disintegration, (pl. V, fig. 13, N). L. Müller regards the formation of colloid material from disintegrated cells as of slight importance. Hürthle remarks that in follicles of mammals with a flattened epithelium, which he considers the final secreting stage, cell remnants or defects in the wall can rarely be found. This is equally true in Teleosts.

I have never seen the signs of degeneration described by Maurer in old carp. He records a swelling of the epithelial cells which breaks down the follicles, permitting lymphatic elements to enter and form lymph nodules, similar to processes in Anura. Perhaps my specimens were not old enough to show these phenomena.

Pigment was not observed within the follicles, though outside of them brown pigment is often found in the supporting tissue. This is probably of haematogenic origin. Baber found brown pigment granules within the colloid in the thyreoid gland of the conger eel. I also fail to find crystals in the follicles or around them as has been reported by some investigators. They are undoubtedly postmortem products.

In the conger eel Baber observed a reticulum between the epithelial cells, in which they were partially imbedded. He states that this reticulum is formed by coagulated intercellular substance and has nodal thickenings. At the thickened places

the 'club shaped' cells described above are located and may be clearly distinguished from the ordinary epithelial cells. I did not find such a reticulum, and it is possible that the filling of the intercellular spaces with colloid substance as before mentioned, may have been what Baber observed. He states that the reticulum (intercellular substance) stained with hematoxylin, which makes it very different from the highly eosinophile network observed by me. Baber's technique, however, seems to have failed to produce the proper differentiation, since he actually succeeds in staining parts of the colloid with nuclear dyes.

The disputed *membrana propria* was not observed. W. Müller, Kölliker and others claim to have seen it everywhere while Schmid and others definitely deny its existence. The connective tissue approaches the follicles and surrounds them but this loose connective tissue sheath, which is by no means always present, could scarcely be called a *propria*.

The blood supply of the thyroid gland is abundant and varies somewhat with the species. Baber is the only observer who has studied the conditions in the Teleost thyroid by aid of the injection method, and unfortunately he used only one specimen of the conger eel.

The capillaries often approach the follicles so closely as to seem imbedded between the epithelial cells. This is best shown when both follicles and vessels are cut in cross section, (pl. IV, fig. 6). Hürthle describes a similar condition in the thyroid glands of young dogs and pictures them in plate II, fig. 6. The epithelial cells often partly surround the capillaries by means of processes, thus forming deep impressions. Baber speaks of small intercellular projections from the capillaries which seem to serve in retarding the circulation of the blood.

There is usually a network of capillaries around each follicle, four or five often being seen in cross section just outside the epithelium, (pl. IV, fig. 5, *a*). In longitudinal section, the capillaries at times surround almost the entire periphery of a follicle. Such specimens illustrate how closely epithelium and endothelium are neighbors without a separating basement membrane, (pl. IV, fig. 5, *E*, *Ca*).

Where the follicles are densely packed, numerous spaces and channels run between them. The smallest of these seem to have no endothelial wall, so that the lymph flows directly against the epithelium of the follicles. In other cases the lymph vessel is indicated by two parallel endothelial lines running between the follicles. This does not agree with L. Müller's view that the blood capillaries are in close contact with the epithelium while the lymph vessels are separated from the follicles by blood vessels or connective tissue. The follicles are sometimes, as described in the anatomical part, situated directly on the big lymph spaces around the ventral aorta as the text figures 4 to 7 show. (See also pl. IV, figs. 2, 3.)

In the conger eel and skate Baber was unable to detect the lymph vessels. Since he injected the venous system which is connected with the lymph vessels he thus regarded the lymph capillaries as veins. Ferguson has been more successful in distinguishing between these two sets of vessels in the dogfish.

In some instances, less often, however, than it occurs in higher vertebrates, a substance was found in the lymph vessels, which had apparently the same structure as the contents of the follicles. The lymph spaces were filled with this substance in one instance and showed many smaller channels running together into the larger ones (pl. V, fig. 18, *L*). According to Anderson the colloid in the lymph vessels undergoes a change, becoming diluted and finely granular and is difficult to distinguish from blood serum.

The way in which the colloidal material leaves the follicle is not made clear by my study. Attention may be called to the varying views of different authors, especially those of Biondi and Anderson, given in their description of the follicular epithelium. It must be mentioned also that Hürthle believes in temporary intercellular channels which form between the cells for the passing of the colloid. I saw in a very few cases a colloidal pseudopodium, as it were, push through the epithelium.

I am also unable to state from the thyreoid gland in the Teleosts whether the veins contain colloidal substances and carry them into the circulation.

## RESUMÉ

The anatomy of the thyreoid gland of the Teleosts is decidedly different from that of most other vertebrates. It is not an anatomical unit. The term 'thyreoid gland,' therefore, is scarcely appropriate. Physiologically isopotent units are distributed over a wide area. Physical influences must be made responsible for this distribution, which is due to mechanical conditions of pressure and pull.

If the thyreoid gland of the Teleosts really have its prototype in the endostyle of the Tunicates, its phylogeny is somewhat as follows. We have at first a uniform organ with a given function, later a change of structure and function takes place, and the organ loses its unity (Myxinoids and Teleosts). In higher forms the new function is maintained but the organ retains its original uniformity and integrity.

The development of the organ from its anlage to the mature state seems to be simpler in Teleosts than in higher vertebrates.

The histology of the glandular elements of the thyreoid in the Teleosts is but little simpler than in higher vertebrates. It shows many parallels to the different features observed by numerous authors in other thyreoid glands.

The function of the thyreoid, concluding from its microscopical appearance, must be closely the same in Teleosts as it is in other vertebrates.

# SPECIAL PART

The species examined were:

ORDER	FAMILY	SPECIES
Apodes	Anguillidae	<i>Anguilla chrysypa</i> .
Isospondyli	Clupeidae	<i>Clupea harengus</i> .
		<i>Brevoortia tyrannus</i> .
	Salmonidae	<i>Oncorhynchus kisutch</i> .
		<i>Salmo mykiss</i> .
		<i>Salmo irideus</i> .
		<i>Cristivomer namaycush</i> .
		<i>Salvelinus fontinalis</i> .
		<i>Osmerus mordax</i> .
Hemibranchii	Argentinidae	<i>Apeltes quadracus</i> .
Lophobranchii	Gasterosteidae	<i>Siphostoma fuscum</i> .
Haplomi	Syngnathidae	<i>Fundulus heteroclitus</i> .
	Poeciliidae	<i>Fundulus majalis</i> .
		<i>Fundulus diaphanus</i> .
Acanthopteri	Atherinidae	<i>Menidia notata</i> .
	Mugilidae	<i>Mugil cephalus</i> .
	Scombridae	<i>Sarda sarda</i> .
	Pomatomidae	<i>Pomatomus saltatrix</i> .
	Serranidae	<i>Morone americana</i> .
	Sparidae	<i>Stenotomus chrysops</i> .
	Sciaenidae	<i>Cynoscion regalis</i> .
		<i>Micropogon undulatus</i> .
	Labridae	<i>Tautoglabrus adspersus</i> .
		<i>Tautoga onitis</i> .
	Tetraodontidae	<i>Spheroides maculatus</i> .
	Triglidae	<i>Prionotus carolinus</i> .
	Batrachoididae	<i>Opsanus tau</i> .
	Blenniidae	<i>Muraenoides gunellus</i> .
	Pleuronectidae	<i>Pseudopleuronectes americanus</i> .

## ANGUILLA CHRYSYPA RAFIN

The thyreoid gland in young eels, 30 cm. long, has a transverse and not a dorso-ventral extension as one might expect in a species with a narrow floor of the pharynx. It begins far forward in the arterial bifurcation lying close under the basihyale and extends back to the second gill arteries (plate I, fig. 11.) Close behind the anterior end of the gland the transverse distribution of follicles becomes rather wide, (fig. 1, A), extending over the



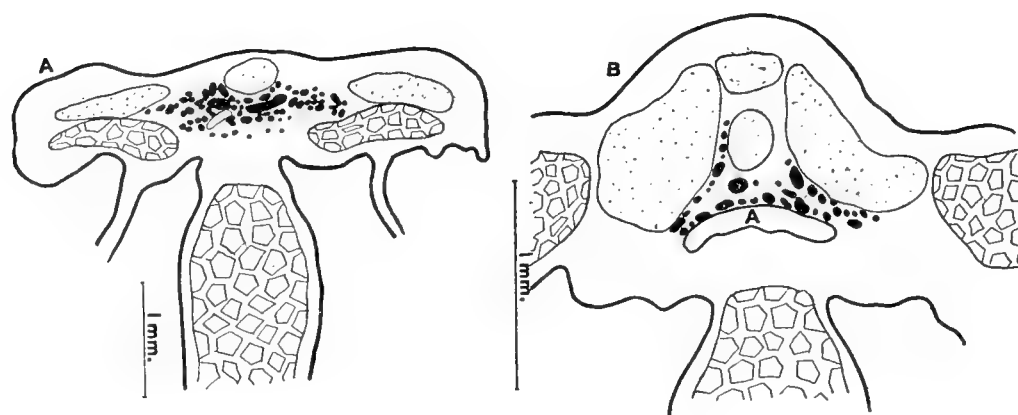


Fig. 1. Sections through the thyroid gland of *Anguilla*. *A*, anterior to the aortic bifurcation; *B*, between the first and second branchial arteries. Thyroid follicles in all figures shown in solid black. Transverse muscles lined. Long-muscles in polygons. Skeletal parts stippled. Arteries heavy line. Veins light line. Lymph sinus broken line. *A*, ventral aorta. *A*<sub>I</sub>, *A*<sub>II</sub>, *A*<sub>III</sub>, branchial arteries.

entire space between the first gill arches, about 2.5 mm. The layer of follicles is very thin so that the dorso-ventral extension is slight. Near the union of the two first gill arteries the follicles are somewhat more dispersed, and reach out dorsally along the sides of the basibranchiale. Some follicles actually lie dorsal to the skeletal parts. The thyroid is in contact with the first gill arteries for a short distance, and here it reaches its maximal extension. Further back it is limited to the neighborhood of the ventral aorta.

Behind the aortic bifurcation the follicles lie closely above and to the sides of the aortic stem and extend along it to the second gill arteries. A string of follicles lies separated between the first and second arterial branches. Baber states that in the conger eel the gland is in the first bifurcation and forms a reddish flattened body. This would correspond to the region of maximal dispersion of thyroid follicles in the species here mentioned.

The follicles exhibit a variety of shapes, elliptical ones being in the majority. They are rather small,  $100\mu$  representing the average diameter of the circular follicles. A few very large follicles are present; these 'giant' follicles as they might be called, are of elliptical shape measuring  $600\mu$  in the long and  $200\mu$  in the

short axis. Baber observed in the conger eel follicles of very large size.

Some follicles send out branches which widen near their end to form secondary cavities, (pl. V, fig. 16). In the series from which one section is figured, (pl. V, fig. 15), may be found a large follicle sending out a branch, and further along two follicles (*F. f.*) connected by a tube (*D*) of high cylindrical epithelium. The tube represents the branch of the former section and in another section both follicles are entirely separated. Going further in the series the small follicle increases in size while the large one sends out a second branch. Thus around a larger follicle as a center may be grouped several smaller ones connecting with the original follicle by 'ducts' as it were. These ducts might be compared with the intercalary portions of other glands. Baber likewise observed branching follicles in the conger eel. Baber claims that new follicles arise from groups of cells somewhat rounded in form and situated in the epithelial wall of the larger ones. I was unable to observe such processes. Lymphatics are present in the thyroid gland of the eel, although Baber denies their existence.

Baber records the follicular epithelial cells as highly columnar in form. I find cuboidal epithelial cells measuring from 10 to 15 $\mu$  high.

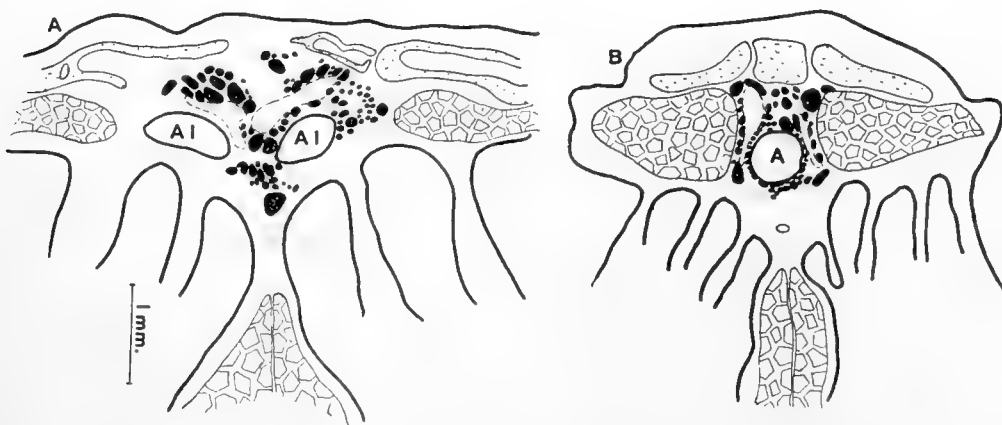


Fig. 2. Sections through the thyroid gland of *Clupea*. A, in the aortic bifurcation; B, between the first and second branchial arteries.

## CLUPEA HARENGUS L.

In the herring (specimens 30 cm. long) the thyreoid gland is well developed (pl. I, fig. 2). The triangular region formed between the floor of the pharynx, bases of the first gill arches and a ventrally lying cartilage is entirely filled with follicles. The distance between the floor of the pharynx and the ventral musculature is considerable, while the cartilages of the basibranchiale are only slightly developed; there is thus sufficient space for a dorso-ventral distribution of the thyreoid follicles (fig. 2, A, B). In certain places the first gill arteries are completely surrounded by follicles; this is also true of the ventral aorta behind the anterior bifurcation (fig. 2, B). Back of the second branchial arteries the extension of the gland diminishes, and only small follicles make a complete ring around the aorta, from which rays of follicles go out towards the cartilages and muscles.

The average size of the follicles is about  $200\mu$  in diameter; very large ones are not seen. The follicular epithelium is in general rather high and varies between narrow cylindrical cells to broad cubical ones. The cells are not very densely arranged. In some regions are found a few neighboring follicles with high epithelial cells which almost obliterate the central follicular space (pl. V, fig. 20, *Coz*). Other follicles have lower cells and all stages exist between these and the normal ones. This suggests a zone of Hürthle's colloid-forming cells. The cytoplasm is highly eosinophile and the nuclei are located near the inner surface of the cells. In the intermediate stages, where there is a lumen in the center of the follicle we find in it colloidal material and red blood corpuscles.

## BREVOORTIA TYRANNUS LATROBE

In this species (length 40 cm.) there are very interesting conditions in the extension of the thyreoid gland, due to the enormous elongation of the gill region. The distance from the heart to the anterior aortic bifurcation measures about 5 cm. and with this stretching of the ventral aorta the thyreoid becomes extended over a long region. The front end of the gland lies well beyond

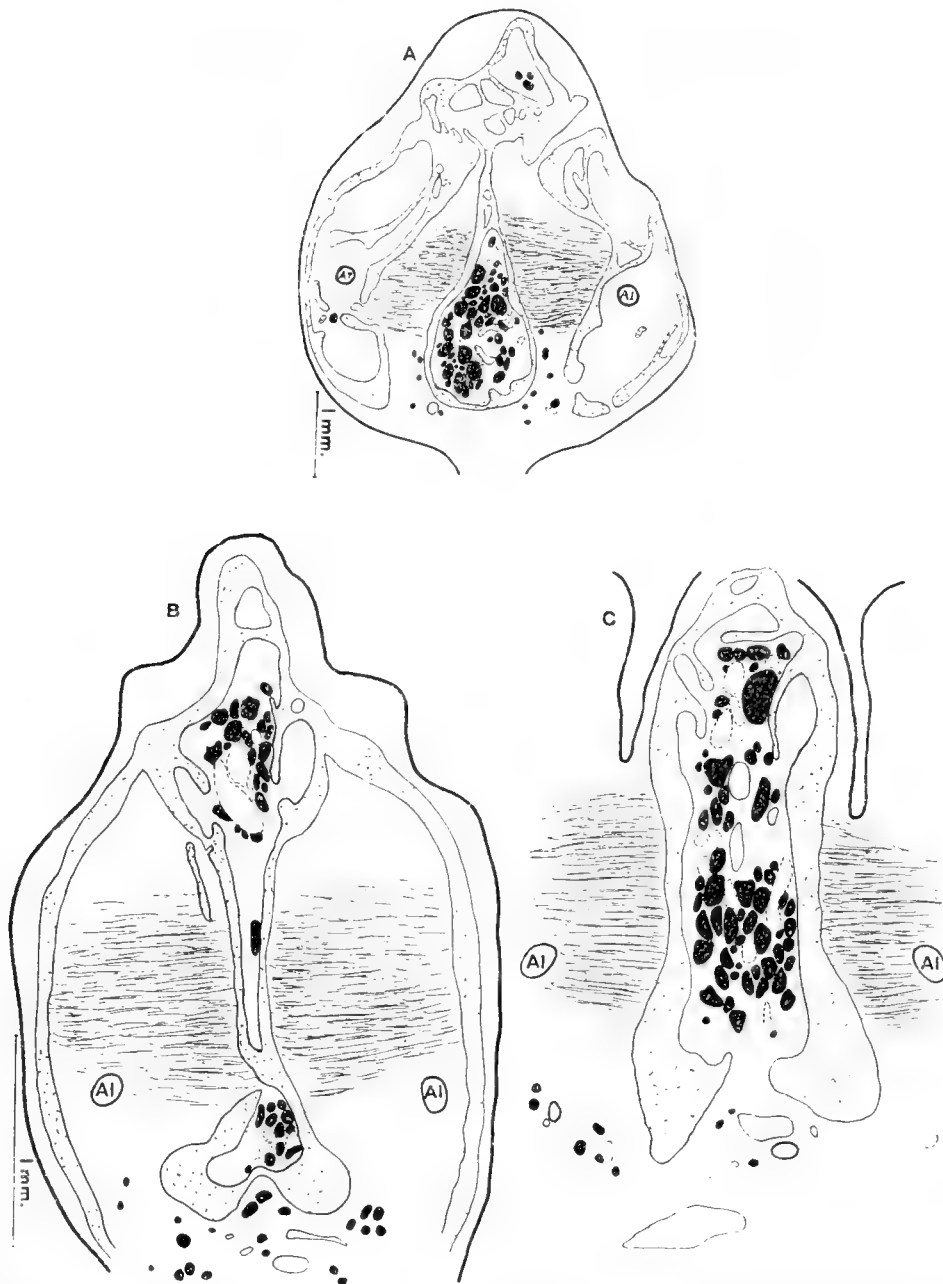


Fig. 3. Sections through the thyroid gland of *Brevoortia*, all anterior to the aortic bifurcation.

the aortic bifurcation and the posterior end is at the second branchial arch, (pl. I, fig. 3).

The floor of the pharynx is very narrow, thus there is no chance for a lateral extension and the thyroid follicles become dispersed only in a dorso-ventral direction. This extension is sometimes 4 mm. high (fig. 3).

The parts of the basibranchiale and hypobranchiale are not compactly developed, the floor of the pharynx being supported by a scaffold of osseous lamellae, between which a wide-meshed fatty tissue appears. There is also an osseous tube open at both ends (representing perhaps a sub-copula) and enclosing the most anterior portion of the ventral aorta (fig. 3, A). In this tube the thyreoid gland extends from the branchial vessels towards the tip of the tongue. In transverse section the gland appears to be lying within a bony ring which completely separates it from the parts outside. At certain places, however, there are openings in this capsule through which the follicles escape into the outside tissue. Within the capsule are found osseous lamellae dividing it into several compartments, and thus three or more bunches of thyreoid tissue may be seen separated by bone.

At the anterior end the follicles lie outside the osseous capsule and are scattered far apart. They are always located in the neighborhood of either blood or lymph vessels and probably follow the vessels as paths of dispersion. The follicles are not always, however, in direct contact with blood vessels.

The osseous capsule lies above the ventral aorta, and we find thyreoid tissue only above the vessel. The first gill arteries for a short distance are completely surrounded by very small follicles. From the aortic bifurcation the follicles extend far forward into the capsule although there are no large vessels, thus there seems to be a tendency towards a forward migration. This is really the only available space into which the thyreoid can expand, unless it enter the ventral musculature.

The histology of the gland is somewhat different from that in other fish. The follicular epithelial cells are drawn out into long processes which come into contact with those arising from the cells of near-by follicles (pl. IV, fig. 1). This suggests that the cells of one follicle might communicate through these processes with those of the adjacent follicles. The only explanation for this phenomenon is as follows: originally the follicles lie close together, with their epithelial cells touching, and when the space between the skeletal parts becomes wider the meshes of the fatty tissue, in which the follicles are suspended, are pulled somewhat

apart, carrying the follicles with them. The cells, which were in contact with others or with blood and lymph vessels may have held fast to them, becoming drawn out into long processes. They thus form a network between the follicles. These bridges often surround the capillaries.

There are only a few follicles which have a regular epithelium with a smooth outline. Outside the bony ring, described above, the follicles have the usual epithelium with a smooth surface.

In places the epithelium was found to be disintegrating. The association of the cells seemed rather loose, their surfaces were also drawn out into long processes like pseudopodia which sometimes divided into two and disappeared in the interfollicular tissue (pl. IV, fig. 5, *E*). These cells did not show any distinction between nucleus and cytoplasm, and their contents was of a foamy nature and showed two or three compact deeply staining granules. They were probably cells which having completed their secretion period were disintegrating.

#### SALMO IRIDEUS GIBBONS

*Specimens 4 cm. long, one month old.* In the young rainbow-trout the thyreoid gland begins in the aortic bifurcation and extends almost to the third gill arteries, (pl. II, fig. 22). There is little space for a lateral extension, as the cartilages of the basi- and hypohyalia form a rather narrow arch, and limit the gland to the space immediately above and below the aorta. At the aortic bifurcation the copula comes close to the vessel, so that the follicles are pressed away from the median line, and lie close to the sides of the cartilage. Later the skeletal parts move back, the space between them becoming somewhat clearer. Half-way between the first and second gill branches the thyreoid gland also extends below the aorta, and a large number of the follicles lie near the second arterial branches. These ventral follicles are smaller than the dorsal ones (fig. 4, *B*). Towards the posterior limit the follicles become smaller and fewer and are again limited to the region above the aorta. Only two or three follicles are seen in a cross section and at the third gill arteries they have entirely disappeared.

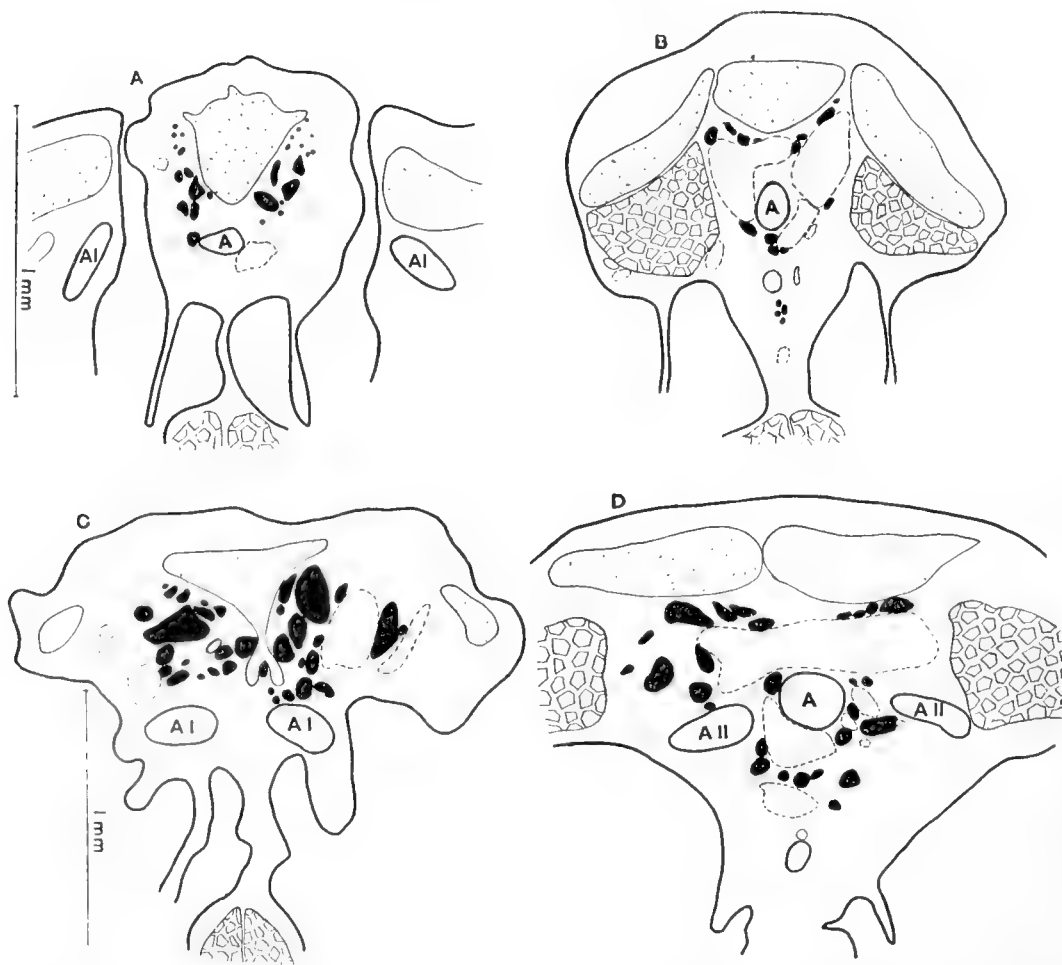


Fig. 4. Sections through the thyroid gland of *Salmo irideus*. *A* and *B*, from a specimen one month old. *A*, just posterior to the aortic bifurcation; *B*, near the second branchial arteries. *C* and *D*, from a specimen one year old; *C*, in the aortic bifurcation; *D*, at the second branchial arteries.

The follicles are usually circular but there are also irregular forms, due to pressure from the surrounding tissues. The diameters of the circular follicles vary between 10 and 80 $\mu$ , the larger ones being rare. The follicular epithelium is everywhere low, the cells measuring about 3 $\mu$ . The nuclei are all placed with their broad side towards the lumen, which might be called the flat cell position to distinguish it from the cylinder cell position in which the nuclei point towards the lumen.

Almost all the follicles are filled with clear homogeneous colloid, which has only here and there retracted a little from the wall. Blood capillaries belonging to the follicles, as observed in other species,

are not visible. Around the aorta there are rather large veins or lymph vessels with extremely thin walls, close to which the follicles lie (fig. 4, *B*). There is no tissue (basement membrane) between the epi- and endothelium, the first being almost as thin as the latter. The nuclei of these epithelial cells are spindle-shaped and lie far apart.

*Specimen one year old.* In this fish the distribution of the thyreoid is about the same as in the younger one, (pl. II, fig. 23). The anterior end is pushed further forward in the aortic bifurcation, and the posterior end still lies close to the third gill branches. The mass of thyreoid tissue is much enlarged. The follicles are much larger in the bifurcation, and in a section there are more than three times as many as in a one month old individual. They are packed more densely and completely fill the space between the cartilages and arteries. The process of the copula mentioned above, which comes down to the level of the aorta, here divides the thyreoid into a right and left half. While in the younger trout the lateral extension of the follicles was less than the dorso-ventral, at this age the floor of the pharynx has become broader through a widening out of the gill arches, and the lateral distribution is more than twice as extensive as the dorso-ventral, although the follicles still go high up along the cartilages (fig. 4, *C*). The follicles also extend some distance along the first branchial arteries. Here the entire thyreoid lies dorsal to the blood vessel and is grouped around two or more large lymph spaces (fig. 4, *C*). Immediately behind the aortic bifurcation the lateral and then the dorso-ventral dispersion of the follicles decrease, so that they lie more densely packed and are fewer in number.

The hypobranchialia approach closer and closer to the copula as we pass backward and force the thyreoid to a more ventral position. Finally the aorta lies almost on the cartilages and the thyreoid shows only one or two follicles in the section. This restriction of the thyreoid zone (pl. II, fig. 23) between the first and second branchial arteries is typical for all salmonids. It may also occur in some other species but is never so pronounced as in the trout.



Near the second branchial arteries the skeletal arch becomes flattened again, the copula does not reach so far down, and first the dorso-ventral and later the lateral distribution of the follicles again increases. Comparatively few follicles now appear below the aorta (fig. 4, *D*). Behind the second branchial arteries the follicles decrease in number and size, and completely disappear before the third branchial arteries are reached.

The follicles are circular, oval or irregular in cross section. The diameters of the circular ones vary between 40 and 200 $\mu$ , the larger ones are more numerous, especially in the anterior region. Branched follicles occur, sometimes as many as five follicles leading into a larger one.

Here also the follicular epithelium is low, almost flat, and the follicles are completely filled with homogeneous colloidal substance. Sometimes, however, the colloid contains particles, probably destroyed blood corpuscles or epithelial cells. The blood supply is rich, many capillaries lying close to the follicles. There seems to be a comparatively better circulation here than in the younger stages.

#### SALMO MYKISS WALBAUM

*Specimen 11 cm.* In the black spotted trout the thyreoid gland shows a great antero-posterior extension. The posterior limit is about that shown by Maurer in a 20 cm. trout, species not named, apparently a brook trout. However, the main part of the gland is situated above the aorta, not below it as Maurer claimed. The anterior limit of the gland lies well in front of the aortic bifurcation and the posterior end behind the third branchial arteries (pl. II, fig. 21). The dorso-ventral distribution is also more pronounced than in most of the other species, especially as to the number of follicles below the aorta. The main mass of the organ lies in the aortic bifurcation (fig. 5, *A*). The copula reaches far down and divides it into two halves. Along this cartilage the follicles extend dorsally close up to the floor of the pharynx. Laterally also the extension of the follicles goes as far as possible.

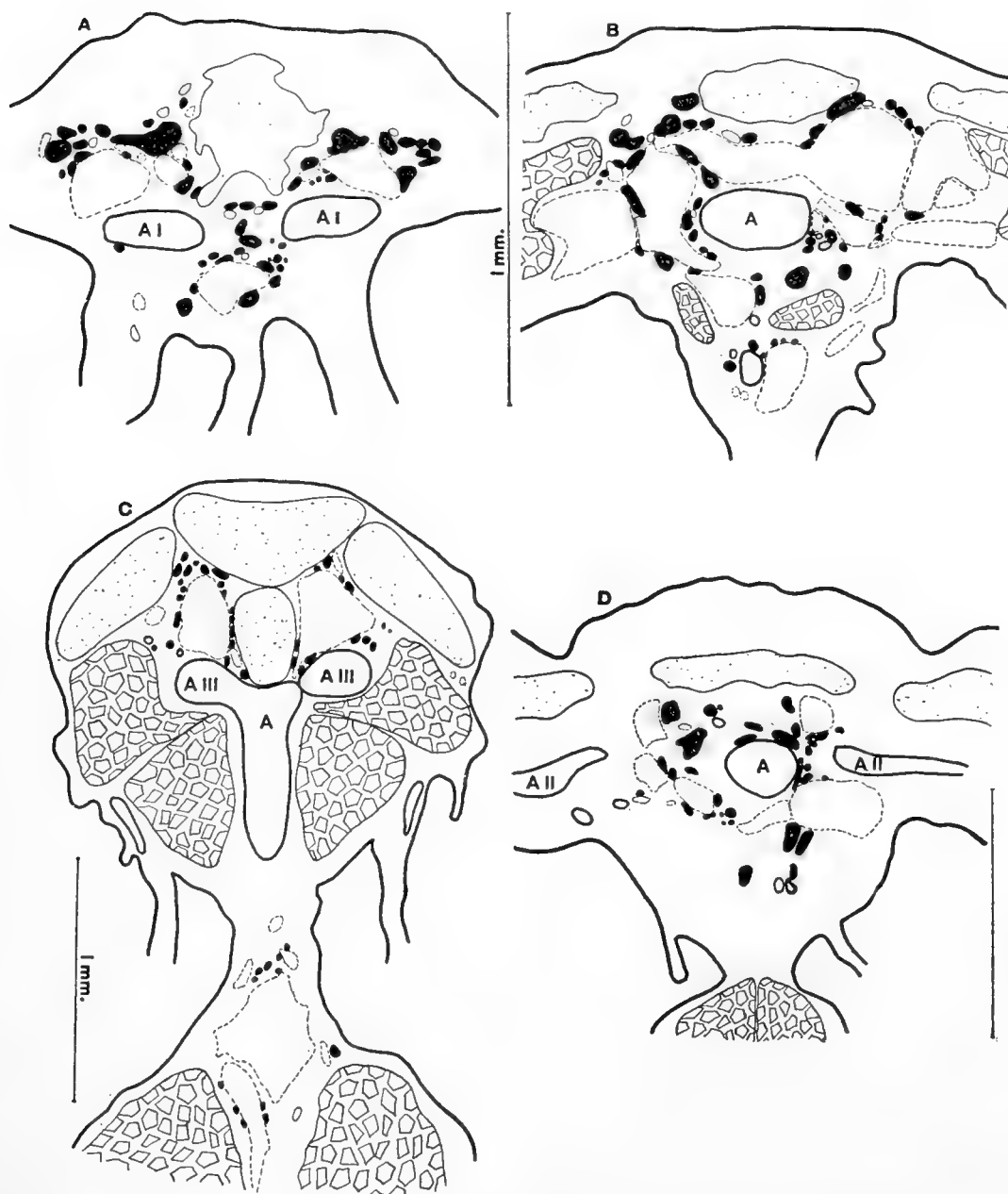


Fig. 5. A to C. Sections through the thyroid gland of *Salmo mykiss*. A, in the aortic bifurcation; B, closely anterior to the second; C, at the third branchial arteries. D. Section through the thyroid gland of *Cristivomer* at the second branchial arteries.

The follicles do not lie directly on the first branchial arteries, but are grouped around large veins or lymph sinuses, the dorsal follicles being much larger than the ventral ones. Behind the first arterial branches the dispersion of follicles is very much reduced. They are forced away from the dorsal region by the

development of the basi- and hypobranchialia and occur only laterally and ventrally of the aorta. Further back even the lateral follicles disappear and only a few small ventral ones are grouped around a small vessel. Space again becomes available towards the second branchial arteries, since the skeletal parts retract more and more, and the follicles reappear in their former locations. The lateral extension however is not as great as in the region of the first arteries, since longitudinal muscle bundles prevent it (fig. 5, *B*). Dorsally the follicles again reach up to the pharyngeal floor. Close to the second branchial arteries the dorsal extension again diminishes and almost disappears when the second gill arteries are reached. Here the follicles lie far below the aorta, as they are forced away from the vessel by a longitudinal muscle. From this place backward a few follicles again appear above the aorta; they are small and scarce, five or six in a section, and widely scattered. Behind the third arteries the ventral aorta lies buried far beneath a muscle, between which and the skeletal parts a portion of the thyreoid lies. Another portion lies below the aorta between the third and fourth aortic branches, and here once more the amount of thyreoid tissue is slightly increased. A small mass of follicles disconnected from the main mass appears behind this place, lying below the aorta.

Cross sections through the follicles are usually circular, some are irregular. Their size decreases from the anterior towards the posterior end of the thyreoid region. The diameters vary between 10 and 60 $\mu$  in a single section. The epithelium is rather flat, though some follicles have a cubical epithelium 3 to 5 $\mu$  high. The nuclei of the flat cells show a peculiar feature; in all other cases they are either round or oval, but here with a few exceptions they are bent, taking forms ranging from wide arches to perfect horse-shoe shapes and are from 8 to 10 $\mu$  long. In all probability they are degenerating, since they do not stain as deeply with nuclear dyes as do the round nuclei. Many of the follicles do not contain colloid.

The blood supply of the thyreoid zone is rich but there are no capillaries to the follicles proper, although there are smaller blood vessels in the region. Large veins and lymph vessels lie around the aorta and the follicles lie close to their walls (fig. 5, *B*).

CRISTIVOMER NAMAYCUSH WALBAUM

*Length of specimen, 12 cm.* The outlines of the thyreoid region in the great-lake trout are about the same as in the former species, but the ventral and posterior extensions are more limited. The anterior end lies in front of the aortic bifurcation, the posterior end at the third branchial arteries, (pl. II, fig. 24). The conditions from the aortic bifurcation to the second branches are the same as described in the species above but at the second arteries the accumulation of thyreoid material is rather large. Here also are found the largest follicles. The lateral extension is wider than at the first branches. The aorta is surrounded by follicles (fig. 5, D) but they do not lie very close to its wall. Posteriorly the extension decreases, three to four follicles being seen in a section above and below the aorta. The ventral follicles soon disappear and at the third aortic branches the dorsal ones also run out.

The follicles are a little larger than those of the black trout. Irregular and circular cross sections of the follicles are seen, the latter 20 to 100 $\mu$  in diameter. The epithelial cells are generally cubical, about 6 $\mu$  high. The nuclei are circular, 3 $\mu$  in diameter, oval or somewhat irregular. The bent nuclei described in the black trout are present, but not so numerous. Some follicles show only regular nuclei, others only irregular, so that one might imagine these forms associated with different physiological stages. Almost all the follicles contain colloid.

There are many capillaries in the fatty tissue in close contact with the follicles. The follicles are not located on large veins and only a few lie close to the lymph sinuses.

SALVELINUS FONTINALIS L.

*Length 4 cm., age 1 month.* In this young brook trout the thyreoid gland has not developed very far, certainly not so far as Maurer describes for this stage. The follicles are scarce, the most anterior lying in the aortic bifurcation. Between the first and second branchial arteries there are a few follicles in each section,

situated above the aorta; near the second a few appear below the aorta (fig. 6, *A*).

*Length 25 to 30 cm.* In the brook trout a condition of remarkably wide distribution of thyreoid material is seen. The region of the thyreoid in this species is comparatively larger than in any other fish. The anterior end of the gland is far in front of the aortic bifurcation and small follicles extend to the floor of the pharynx (fig. 6, *B*).

The first branchial arches are completely surrounded by thyreoid follicles. In the aortic bifurcation the follicles are very numerous, densely packed and occupy a rather large field. They reach up to the dorsal edge of the copula and laterally to the gill bases. On both sides of the aorta they are scattered between the fibres of longitudinal muscles (fig. 6, *C*). The follicles force their way through the muscle tissue along blood vessels and connective tissue fibres. Below the aorta their arrangement is less dense. Close behind the aortic bifurcation the amount of thyreoid tissue is reduced in the typical way, the copula extending down to the aorta. By this arrangement three, more or less separated, thyreoid masses are formed, two dorsally to the right and left of the copula and one below the aorta. The ventral part decreases, then the dorsal masses, the arrangement of the follicles becoming looser. Although the dorsal space becomes more open the follicles still decrease in size and are scattered far apart, indicating that this is a zone between two accumulations of thyreoid tissue, those around the first and second aortic branches. Two centers of growth may easily be determined.

Just before reaching the second branchial arteries the lateral extension becomes very great (fig. 6, *D*). The follicles migrate into the first gill arches along the branchial arteries and occur at the base of and extend into the second gill arches. This wide distribution of thyreoid elements is certainly the most remarkable feature of the organ in the Teleosts. Follicles not only lie at the base of the gills, but are distributed along the laminae at the base of the villi.

At the second branchial arteries the thyreoid gland, as mentioned above, once more shows an extensive development. Above

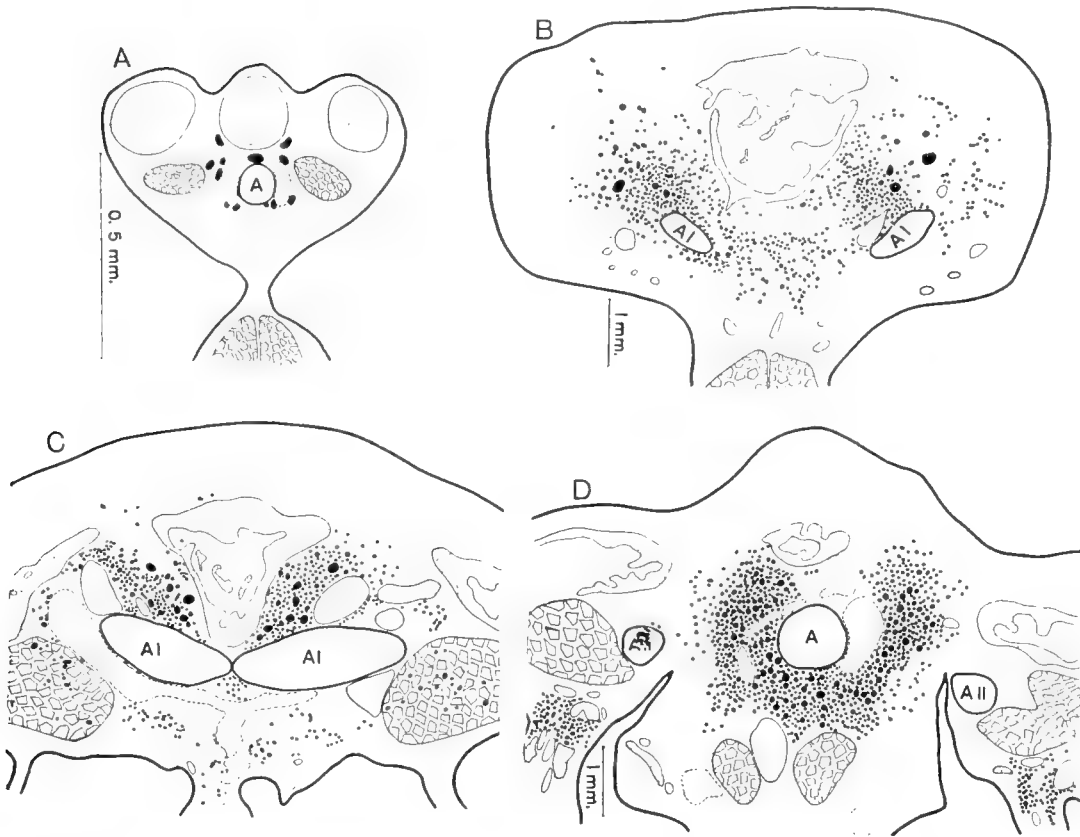


Fig. 6. A. Section through the thyroid gland of a one month old specimen of *Salvelinus fontinalis*, between the first and second branchial arteries.

B to D. Sections through the thyroid gland of an adult specimen. B and C, in the aortic bifurcation; D, near the second branchial arteries.

the aorta a dense arrangement of follicles is seen and below the number increases. Laterally the follicles extend along the arteries. Behind this place the aortic stem is entirely surrounded by thyroid tissue which completely fills the space between bones and muscles. Small follicles are imbedded in the adventitia of the aorta. There is a dissolution of the dense arrangement in the peripheral zones, especially ventrally. Close behind the second branchial arteries the mass of thyroid tissue decreases very suddenly and only a thin ring of follicles surrounds the aorta. Towards the third branchial arteries the aorta becomes buried between the ventral muscles, and the ventral follicles disappear sooner than the dorsal ones which continue and surround the third branchial arches for a short distance. Behind the third

branchial arteries a small accumulation of thyreoid tissue once more appears.

A second series shows conditions similar to those above described. The follicles in the anterior region are less densely arranged. The basibranchiale comes very close to the aorta and separates to some extent two portions of thyreoid tissue along the aortic stem. The follicular mass is little reduced behind the aortic bifurcation but a little in front of the second branchial arteries the typical restriction is found. At this place the first ventral follicles appear. When the second aortic branches are reached the lateral extension of follicles becomes very wide. The mass of thyreoid tissue is here much increased, and the ventral portion is well developed but not so far as in the trout described above. The thyreoid stops close behind the second branchial arteries.

In a third series the separation of follicles in the anterior portions is still greater than described in either the first or second. The dorsal limit reaches to the upper edge of the basihyale, where there is an accumulation of follicles on both sides. The first branchial arteries are for a long distance completely surrounded by follicles, but the number of follicles decreases visibly towards their union; thus in this case there is an accumulation of follicles in front of the first aortic bifurcation. The ventral follicles appear at the first branchial arteries and disappear before reaching the second. It seems that here the entire thyreoid mass is pushed much farther towards the head than in the other trout described. Between the first and second branchial arteries the conditions are similar to those in the other specimens, the distribution of the follicles being restricted. There is no pronounced increase of thyreoid tissue or lateral distribution at the second branchial arteries and the posterior limit of thyreoid follicles is in front of the third branchial arteries.

High epithelial cells were predominant in the follicles of all the thyreoids. The cubical cells measure 9 to 10 $\mu$  broad and 12 $\mu$  high, and the narrow cylindrical cells are 2 to 3 $\mu$  broad and 20 $\mu$  high. The nuclei are usually large and round, except in the very high cells where they are compressed. In a few places

not all the nuclei of a follicle show the same structure or the same reaction towards the stain and thus may be in different physiological stages. In addition to normal, large nuclei with distinct nucleoli and granular structure we find compact deeply staining nuclei which sometimes contain a vesicle. There are also small pyknotic nuclei in small (degenerating) cells. Often such compact nuclei with a halo of colloid are found within the lumen and it seems then that the epithelial cells have emptied their entire content. These masses can be easily distinguished in the colloid even after their outlines become indistinct as they have a different refractive index. Maurer describes somewhat similar structures in trout and carp. In other cases several neighboring cells with much swollen bodies have been pushed off from the epithelium and may be seen in the colloidal substance (pl. V, fig. 13).

The general form of the follicles is globular, though the surrounding fat and muscle tissue influences the outlines to some degree (pl. V, figs. 10-12).

Smooth muscle fibres are found in the entire thyreoid region; in one case (the first specimen) only ventral to the aorta. They run in all directions in the interfollicular tissue. The follicles are often arranged along them or are surrounded by them. Where the follicles lie in clusters of five or ten or more, smooth muscle fibres are found running between them. The muscle fibres with the follicles, their capillaries and the connective tissue fibres form a somewhat compact structure.

The blood supply to the secreting epithelium is extremely rich, several capillaries going to each follicle (pl. V, figs. 10, 12 *Ca*).

The thyreoid gland in two other species was dissected out as far as it was visible macroscopically. In this way of course one does not get the scattered follicles but only the main masses. Figs. 28 and 29 of plate III from these two dissections as well as figs. 25 to 27 of plate II, which are from specimens cut in serial sections, show that the distribution of the thyreoid in the trout is very variable.



## ONCORHYNCHUS KISUTCH WALBAUM

*Specimen 6 months old, 7 cm. long.* The thyroid gland in the silver salmon extends further back than in most of the trouts, reaching beyond the fourth branchial arteries (pl. V, fig. 20). Another feature in the arrangement is that the follicles lie rather close together, surrounding the stem of the ventral aorta through-



Fig. 7. Sections through the thyroid gland of *Oncorhynchus*. A, just posterior to the aortic bifurcation; B, at the second branchial arteries; C, posterior to the third branchial arteries.

out almost its entire length. The amount of thyroid tissue is small at the aortic bifurcation and between the first and second branchial branches (fig. 7, A). At the second gill artery the thyroid tissue is most abundant.

In front of the aortic bifurcation the basi- and hypobranchialia reach down and here only a few follicles are found on both sides of the hypohyalia. Back of the place where the cartilages have retracted the longitudinal muscle bundles prevent the lateral expansion of the thyreoid (pl. V, fig. 14). At the second branchial arteries however, the mass of thyreoid tissue is very much increased, again surrounding the aorta (fig. 7, *B*). The ventral extension is pronounced. As a rule in the trouts no follicles lie directly against the aortic wall but here there is a complete ring of them around it. Above this ring lies a large lymph sinus and between it and the skeletal parts thyreoid tissue is again found. Towards the third aortic branches the cartilages again compress the aorta, and here the follicles lie around the aorta and along the outlines of the cartilages. Further back the aorta sinks down between the muscles, the ventral follicles disappear and the dorsal ones do not follow the vessel, but increase in number and group themselves around a subcopula between the third branchial branches. This dorsal rather compact group of follicles extends back behind the fourth branchial arteries. Below the aorta a small cluster of four or five follicles appears as is seen in other species of trout (fig. 7, *C*). At the level of the fourth aortic branches the copula extends so far ventrally that the dorsal follicles are pressed between the muscles and again come down into contact with the aorta. The posterior end of the thyreoid is in this region of the fourth arch.

The form of the follicles is usually elliptical, though circular cross sections are also found, ranging from 15 to 100 $\mu$  in diameter. The larger ones are more abundant.

The follicular epithelium is very flat (pl. V, fig. 14), and in most of the cells are again seen the irregular nuclei described in some of the above species of trout (pl. IV, fig. 9, *N*). The majority of follicles are in close relation with large lymph sinuses, epithelium and endothelium being in contact (fig. 7, *B*, *C*). There are no capillaries to the follicles proper.

## OSMERUS MORDAX MITCHILL

A smelt 20 cm. long. This smelt presents the thyroid conditions described below. A few follicles appear far in front of the aortic bifurcation (fig. 8, A), and further back more are arranged around the copula. At the bifurcation every section shows twelve to fifteen follicles between the stem of the aorta and the

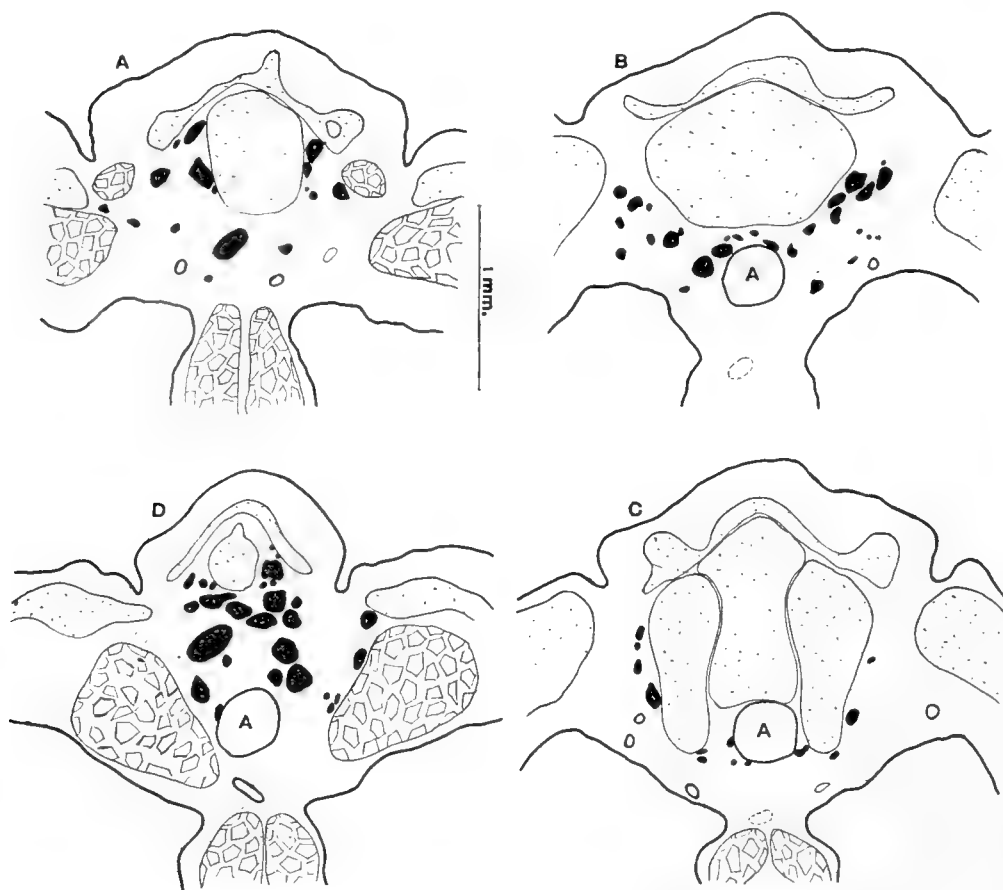


Fig. 8. Sections through the thyroid gland of *Osmerus*. A, anterior to the aortic bifurcation; B, just posterior to it; C, between the first and second; and D, close to the second branchial arteries.

copula. The follicles vary in size from 40 to 200 $\mu$ . Behind this the main mass of thyroid tissue lies above the aorta, a few follicles lie to either side, and ventral to the aorta they are very scarce. Further back the basibranchiale comes nearer and nearer the aorta, finally reaching it, so that the follicles are forced out laterally (fig. 8, C). In the region of the hypobranchialia are

seen only a few follicles far to the sides of the aorta and skeletal parts. Behind this the two hypobranchialia have retracted a little from the copula and very small follicles appear in the crevices between them. As the copula retracts from the aortic stem, more follicles appear on the dorsal side of the aorta. At the second branchial arteries the follicles cease (pl. I, fig. 4). The follicular epithelial cells are low cuboidal with the longer axis parallel to the base. The colloid appears homogeneous.

#### SIPHOSTOMA FUSCUM STORER

*Specimen 30 cm. long.* In the pipe-fish the thyreoid gland consists of entirely isolated follicles, lying above and to the sides of the aorta (fig. 9). The external form of the fish influences, of course, the form of its inner organs. The thyreoid gland has not found room for dorsal, ventral or lateral expansion and therefore extends far backwards as a rather narrow streak. The anterior end lies at the aortic bifurcation and the posterior end close to the bulbus arteriosus (pl. I, fig. 5). Thus we have a condition in which the organ reaches further towards the tail than usual and where the thyreoid region tapers towards the head end, while as a rule the reverse is true. The number of follicles is not very large, five or six to the section behind the aortic bifurcation. The number decreases towards the second branchial arteries and still more so towards the third, where a transverse muscle occupies the space between the bones and the aorta. At this place there are only one or two follicles in a section, yet there is a continuous chain of them. Near the third branchial arteries the aorta goes down ventrally, the transverse muscle has decreased, and thus the thyreoid finds more space for development. There are six or eight follicles in a section and they lie between the third gill branches which run dorso-laterally. Behind this place the dispersion of follicles increases (pl. IV, fig. 7), although the aorta lies far ventrally, a fact showing that the thyreoid follicles do not necessarily use the aortic stem as a migration path. On each side of the median line a muscle runs in an antero-posterior direction upon and under which the thyreoid follicles lie. The ventral

group consists only of small follicles which have traveled downwards along a vein running between the two halves of the muscle. Further back the muscle bundles separate and here the greatest mass of thyroid tissue is found. The space between the pharynx and bulbus is well filled by follicles which lie in a chaos of capillaries (pl. IV, fig. 7, *Ca*, *F*).

Histologically this gland is as different from that in other species as it is anatomically. The gland, when fixed, may have been

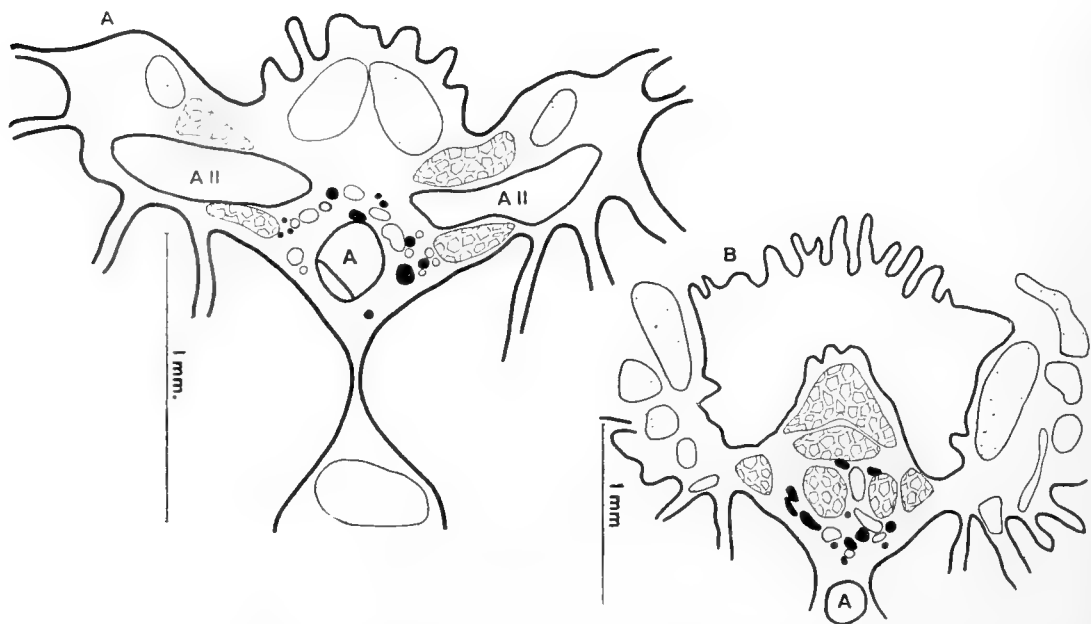


Fig. 9. Sections through the thyroid gland of *Siphostoma*. *A*, at the second; and *B*, posterior to the branchial arteries.

in a peculiar state of function since there are no reasons to assume that the histological structures observed are permanent. Collöid was not found in any of the follicles, at least not as a uniformly compact mass. Certain follicles contained highly acidophile lumps about the size of epithelial cells. The epithelium, however, seemed in a state of colloidalization. The cells were high, cuboidal and swollen, with bulged out bases and surfaces (pl. IV, fig. 8). The nuclei were centrally located or towards the lumen. Thus they seemed to be typical colloid forming cells. The nuclei are in some cases round and massive, usually however they are very irregular. In some it seemed as though amitosis was taking place.

The formation of colloid ordinarily occurs in only a part of the thyreoid at a time. Here, however, the entire gland seemed to be in a similar physiological state.

FUNDULUS HETEROCLITUS L.

*Specimens 10 cm. in length.* The follicles in the region of the aortic bifurcation are grouped around a vein, most of them lying to the sides of it and under a transverse muscle. The elliptical shape of the vein in sections indicates the pressure between this muscle and the m. sternohyoideus which forces the follicles out from the median line. The follicles become more numerous towards the aortic bifurcation and they extend part way out along the first branchial arteries, and more on their ventral than dorsal side. Between the first and second gill branches follicles are found under and above the transverse muscles around which they have traveled. The ventral aorta in this region is completely surrounded by thyreoid tissue, more being found on the sides than either dorsally or ventrally (fig. 10, B). At the second gill branches the follicles again spread out laterally. Behind this place only a few scattered follicles are found (pl. I, fig. 6).

The size of the follicles varies extremely. The smallest are found at the anterior end and the largest in the middle of the thyreoid region. They are either circular in cross section, oval or with irregular evaginations.

The epithelial cells are usually cubical, but in very small follicles sometimes columnar, while in large empty follicles the cells are flat. Narrower cells with spindle shaped nuclei are seen in places.

The colloid is granular, and in some regions is seen to leave the follicle. Whether this is due to artificial pressure cannot be stated. Occasionally two neighboring epithelial cells will flatten out somewhat as if they were about to form a passage between them.

The blood supply to the thyreoid region is rich. The follicles are almost completely surrounded by a net of capillaries. These vessels are so pressed against the follicle that they form grooves in it (pl. IV, fig. 6). The projections of the epithelium between

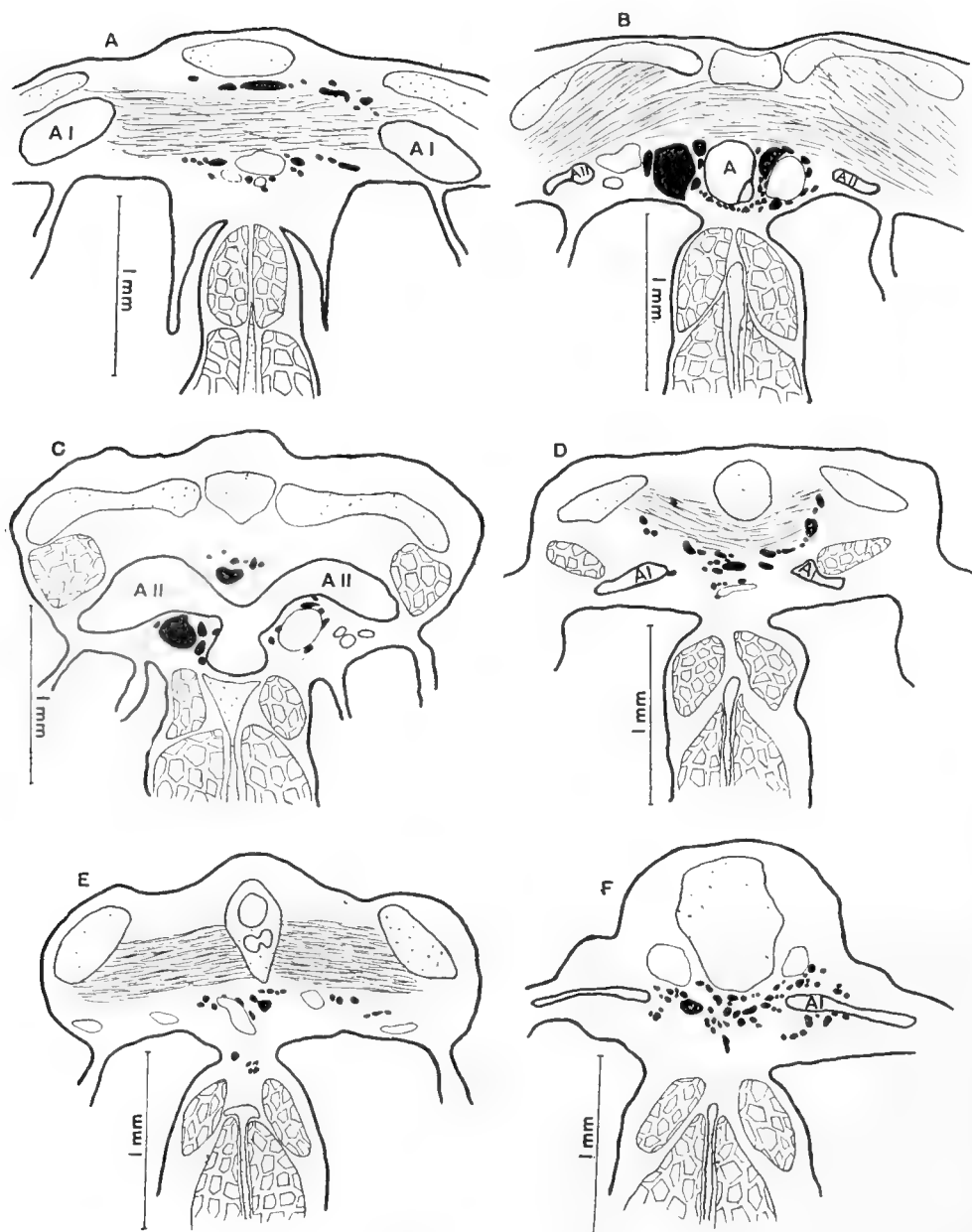


Fig. 10. Sections through the thyroid gland of *Fundulus*. *A* to *C*, *F. heteroclitus*. *A*, in the aortic bifurcation; *B*, anterior to; *C*, at the second branchial arteries. *D*, *F. diaphanus*, in the aortic bifurcation. *E* and *F*, *F. majalis*, anterior to the aortic bifurcation.

the capillaries show narrower and longer cells, and some of these cells entirely lose their communication with the follicular lumen. The connective tissue sometimes forms an almost complete sheath around the follicles and their capillaries. Red blood corpuscles in all stages of disintegration are found in many follicles

(see General Part). How these corpuscles get into the lumen could not be determined. Erythrocytes are often seen partially imbedded in the follicular epithelium as if they would force their way in between two cells. In other places corpuscles are found pressed against an epithelial cell which has so flattened out that only a thin layer of cytoplasm separates the corpuscle from the lumen.

FUNDULUS DIAPHANUS LE SUEUR

*A specimen 9 cm. long.* The main mass of the thyreoid is located a little nearer the tip of the tongue than in *F. heteroclitus* (pl. I, fig. 7). The posterior end lies at the second branchial arteries where the follicles become scarce and scattered. A further difference from *heteroclitus* is that the main mass of follicles always lies above the aortic stem, only a few small ones lying below. The lateral extension is here also unimportant.

The floor of the pharynx is narrow and the connection between it and the ventral musculature is only a narrow streak. In *heteroclitus* the lateral pharyngeal axis is the longer one, therefore, the lateral thyreoid extension prevails; while in *diaphanus* the dorso-ventral axis is longer, and here the extension of the thyreoid is mainly in this direction. Ventrally, however, it is prevented by the narrow isthmus, and follicles are mainly found above the aorta (fig. 10, *D*). In this way the distribution of the follicles may be figured out mechanically in almost every case.

The follicles are of all sizes, though not so large as in *heteroclitus*. There are more elliptical or irregular ones and these have a longer axis. The cuboidal cells of the follicular epithelium are not as high as in *heteroclitus* and cylindrical ones are not found. The colloid is homogeneous and the blood supply is not rich.

FUNDULUS MAJALIS WALBAUM

*Length of specimen 9 cm.* The follicles spread out laterally much further than in the other two species (fig. 10, *E, F*). They extend for a distance along the first aortic branches. Between the first and second branches there is only a narrow streak of thyreoid



tissue, but the main mass of the organ lies at the second gill branches and here the greatest lateral extension occurs under a transverse muscle. The vertical extension is small and there are no follicles below the aorta. Behind the second gill branches is found the posterior limit of the gland (pl. I, fig. 8).

The follicles are still smaller than in *diaphanus* and more uniform in size. The circular type predominates and they are more numerous than in the other species. The colloid is homogeneous and the follicular epithelium similar to that in *diaphanus*.

#### MENIDIA NOTATA MITCHILL

*Length of specimen 10 cm.* The thyreoid mass is rather small (pl. I, fig. 9). The follicles are extremely small,  $20-25\mu$ , and are scattered along the stem of the aorta between the first and second branchial arteries and out along the second arteries. The lateral extension is greater than the antero-posterior.

#### MUGIL CEPHALUS L.

*A mullet 15 cm. long.* Small follicles are found in the anterior end of the thyreoid region and are grouped around a vein (fig. 11, A). At the aortic bifurcation the organ is better developed, but is hardly in contact with the gill vessels (fig. 11, B). The thyreoid lies above the aorta, and at the second branchial arteries it comes into contact with the vessel. Here the gland is well developed with numerous large follicles. The follicles disappear towards the third aortic branches (pl. I, fig. 13).

The size of the follicles varies between  $30$  and  $140\mu$ . In section they are slightly oval. In the follicular wall are found transitions from flat to high epithelium. The height of the cells varies within the same follicle, showing that it is independent of follicle size. The height of the cells rather depends upon outside pressure, *e.g.*, a follicle pressed into oval shape by cartilage shows low epithelium on the longer sides and higher cells on the short sides.

SARDA SARDA BLOCH

*Length of specimen 50 cm.* The thyreoid gland of the Spanish mackerel shows the most remarkable conditions of all fish thyreoids.

The mass of the organ is enormously large and the dorso-ventral and cephalo-caudad extensions are unusual. The relation of the thyreoid gland to other tissue is singular, and could be compared only with that in *Brevoortia*. There exists such an intermingling of thyreoid, bone, cartilage, smooth and striated muscle fibres, fat and connective tissue that it is impossible sharply to define

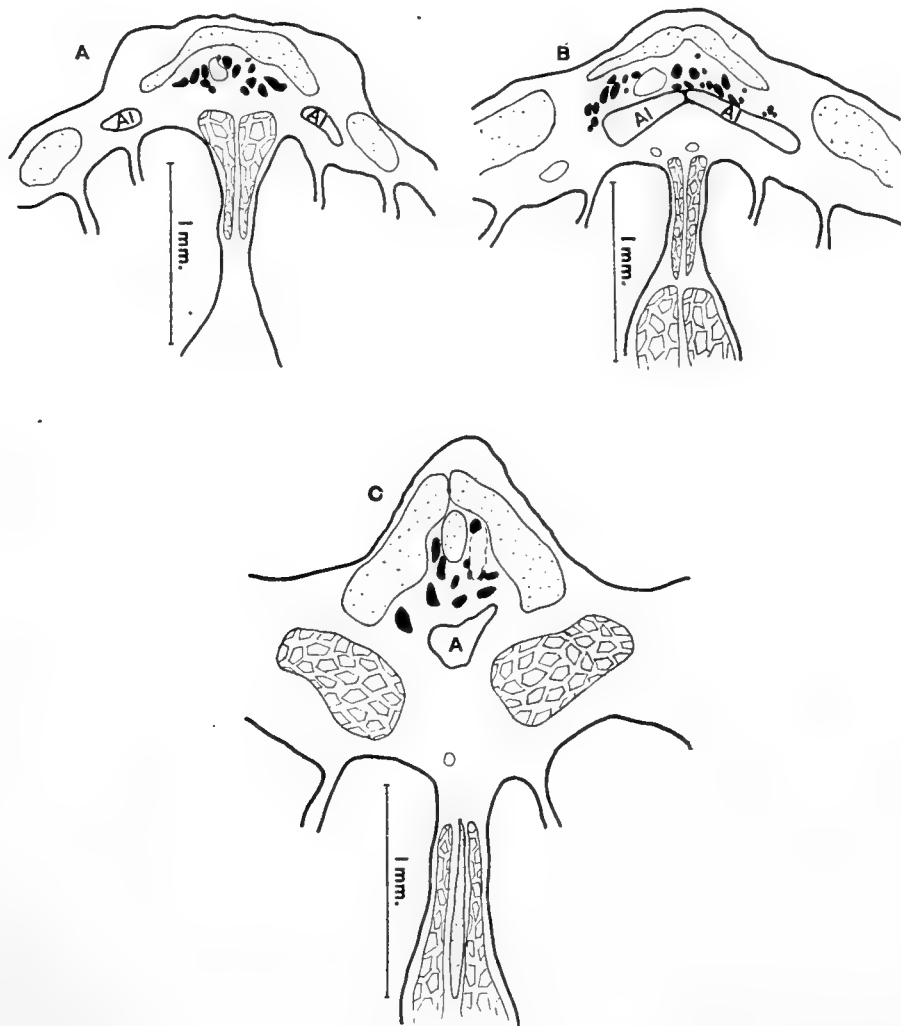


Fig. 11. Sections through the thyreoid gland of *Mugil*. *A*, anterior to; *B*, at the aortic bifurcation; *C*, near the second branchial arteries.

the organ. Yet on the other hand, there can hardly be found a group of follicles detached from the main thyreoid body.

The isthmus is long, as in *Brevoortia*, and hence the thyreoid region is much elongated (pl. I, fig. 10), measuring 4 cm. in length. It is not, however, as narrow as in the menhaden, having a wide lateral extension. The anterior end is pushed far forward, 2.5 cm. in front of the aortic bifurcation, so that it also comes to lie in front of the hyoid arch. The entire development of the organ takes place more cephalad than usual and the main mass lies in front of the aortic bifurcation (sharks!), deeply buried in the body of the tongue, as a consequence of the ventral extension of the copulo-hyoid (fig. 12, *A*). It occupies a more ventral position than any other fish thyreoid. The follicles are located around a large vein and are rather closely arranged. As the basi- and hypohyalia retract the follicles creep into the clefts between them and thus the thyreoid mass assumes the shape of a horse-shoe, the two arms of which point dorsally (fig. 12, *B, C*). The smooth muscle fibres of this region are completely invaded by follicles (pl. IV, fig. 21), as are also the bones of the gill arch, especially the copula, in regions where they lose their compactness and break up into lamellae. The thyreoid takes the form of three masses converging ventrally, and as we pass back it expands more and more on the sides, 6 to 7 mm., while the median branch becomes smaller. About one cm. in front of the aortic bifurcation the most extensive region of the gland is reached. In cross section the mass is rhomboidal, the diagonals being about 7 and 4 mm. The lateral extension decreases while the ventro-median mass increases, from which two branches tend dorsally along the edge of the copula. Thus again the sections show a horse-shoe shape, with a broad middle piece and narrow dorsally converging arms, in which the follicles are oval with their longer axis parallel to the line of extension. On reaching the first branchial arteries, which run in this species towards a ventro-lateral zone and do not come into contact with the follicles (fig. 12, *C*) we pass to their union where a few follicles surround them (fig. 12, *D*). The central portion of the gland becomes smaller and lies separate in the aortic bifurcation while

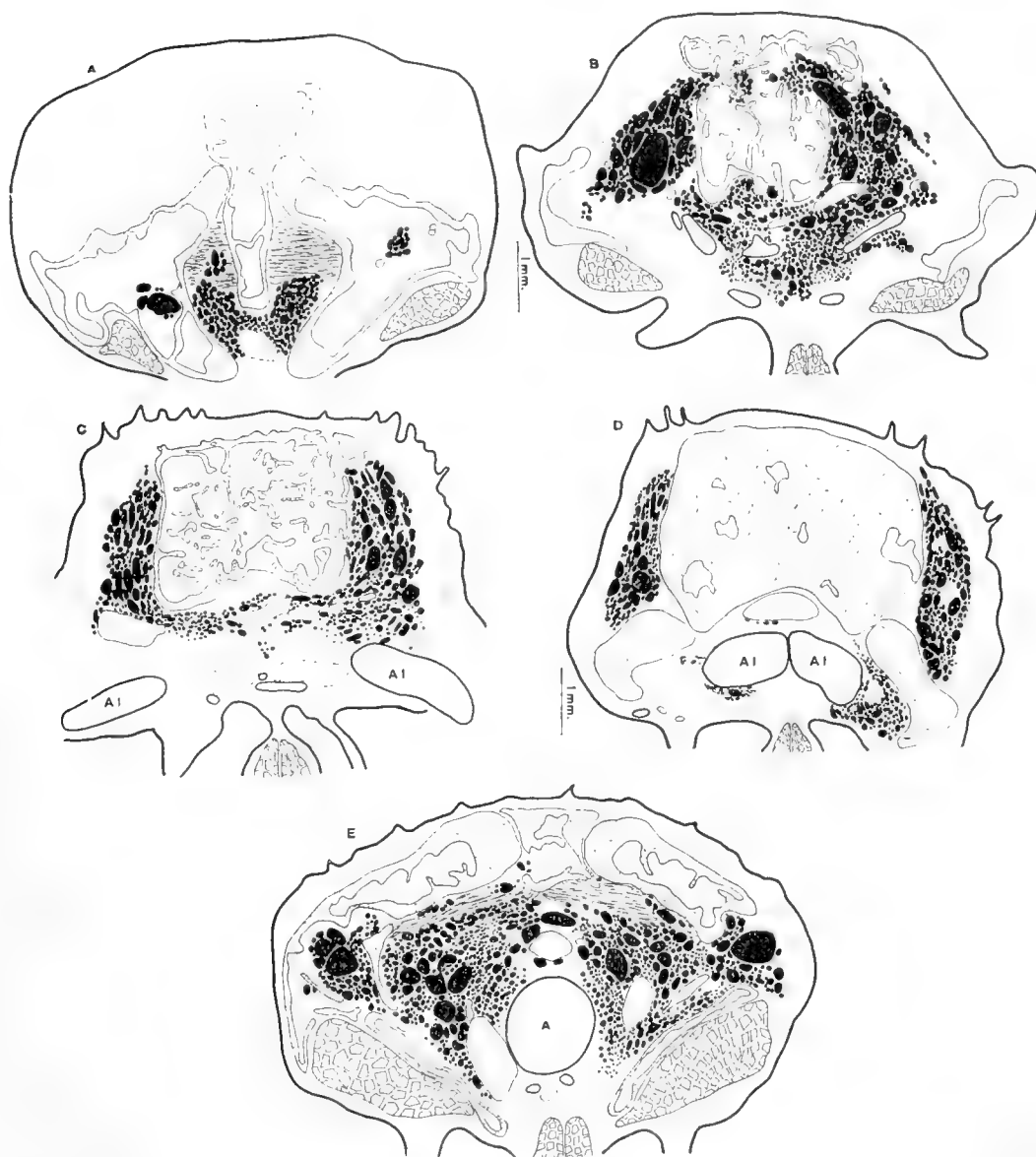


Fig. 12. Sections through the thyroid gland of *Sarda*. *A*, near the anterior end of the gland; *B*, in the region of greatest extension anterior to the aortic bifurcation; *C* and *D*, close to the aortic bifurcation; *E*, near the second branchial arteries, the region of greatest extension.

the lateral parts increase posteriorly. Thus in the sections there are three portions of thyroid which become more and more separated by the enlargement of the copula (fig. 12, *D*). Behind the aortic bifurcation some follicles appear below the aorta; the middle mass again enlarges and the three parts unite. One branch again extends into the copula and soon becomes smaller, while the lateral

portions increase. The posterior end of the gland is found behind the second gill branches.

The follicles are usually circular or oval in cross sections, though many are polygonal from pressure. Their size varies between 30 and 350 $\mu$  medium sizes being most abundant. Giant follicles reach 800 $\mu$  long by 400 $\mu$  in short diameter. Many follicles are without colloid, while in others the colloid is much more shrunk than usual. The colloid is homogeneous. The follicular epithelium is of high cylindrical cells or cubical ones. The cytoplasm is stained more darkly in the basal portions; in the higher parts it is sometimes reddish. The blood supply is rich.

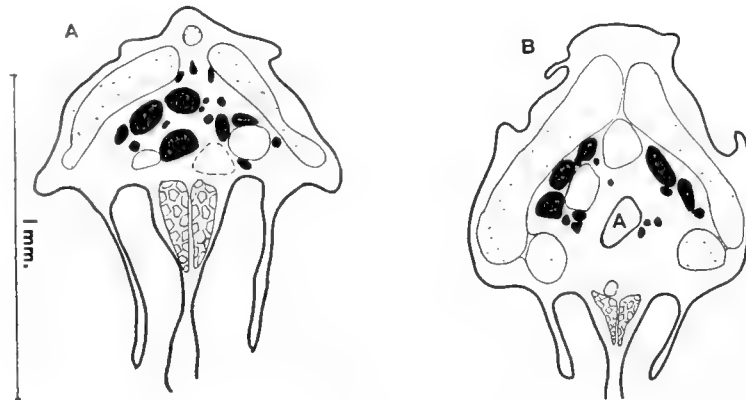


Fig. 13. Sections through the thyroid gland of *Pomatomus*. A, anterior to the aortic bifurcation; B, between the second and third branchial arteries.

#### POMATOMUS SALTATRIX L.

*Young bluefish 30 cm. long.* In this species the dispersion of the thyroid follicles is prevented in both a lateral and dorso-ventral direction, since the arch formed by the basibranchiale and hypobranchialia is very narrow (fig. 13). The gland is thus a long narrow streak (pl. I, fig. 11). At the aortic bifurcation there are only a few follicles, some of which lie close to the first gill arteries, just in front of their point of union. The thyroid mass reaches its maximum extension above the ventral aorta and between the first and second gill arteries, especially towards the second. But

even here there are only ten or fifteen follicles in a cross section. Some follicles lie close to the base of the second gill arteries and from this point the gland extends, with from six to ten follicles in a cross section, to a little behind the third branchial arteries where it ends. The follicles are generally dorsal to the ventral aorta (fig. 13, *B*), only a few being below it.

The form of the follicles is irregular, but approaches the globular type. Their size ranges from 15–100 $\mu$  in diameter though some are far above this size (giant follicles). The minute histology shows no peculiarities. The epithelium is usually cubical, the cells being 6 $\mu$  high.

#### MORONE AMERICANA GMELIN

*Specimen 35 cm. in length.* The thyreoid gland of the white perch is characterized by the enormous size of nearly all the follicles as well as by their unusually loose arrangement. Cephalad of the aortic bifurcation there is little room for dispersion since the copula reaches far down and the skeletal arch is rather narrow. Behind the bifurcation (fig. 14, *B*) this arch becomes wider and from here to the second gill arteries the main mass of the thyreoid is situated (pl. I, fig. 12). From the second branchial arch towards the third two narrow lines of follicles run along the sides of the aorta. The entire length of the thyreoid region measures 3.5 cm. The majority of the follicles lie above the aorta except in the anterior region.

The size of the follicles varies from 120 to 600 $\mu$  in diameter, the very large ones are most abundant especially in the more anterior region. In cross sections the follicles are almost all circular. The epithelial cells are low, 3 to 4 $\mu$  high. In these follicles there are no indentations in the colloid, it either fills out the lumen completely or is retracted from the epithelium and has a smooth edge. (The differences in the colloid of different species may be of some physiological significance.)

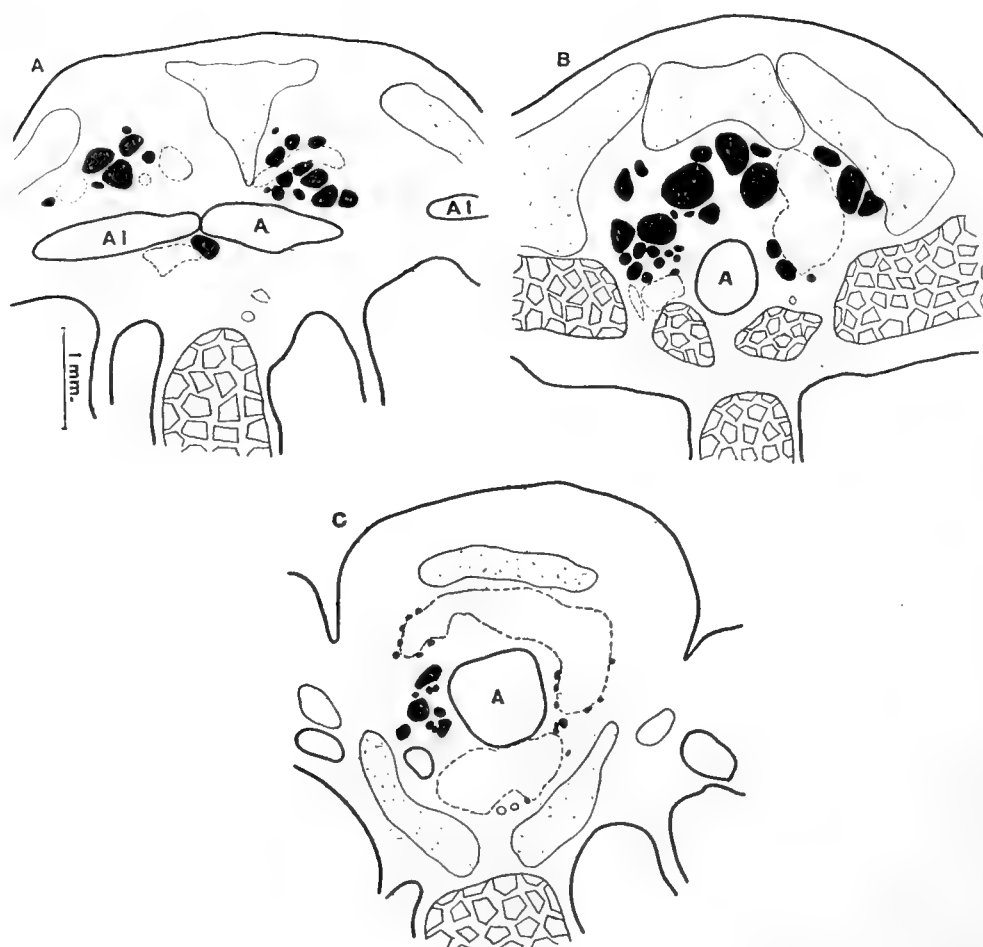


Fig. 14. Section through the thyroid gland of *Morone*. *A*, at the aortic bifurcation; *B*, between first and second; *C*, close to the second branchial arteries.

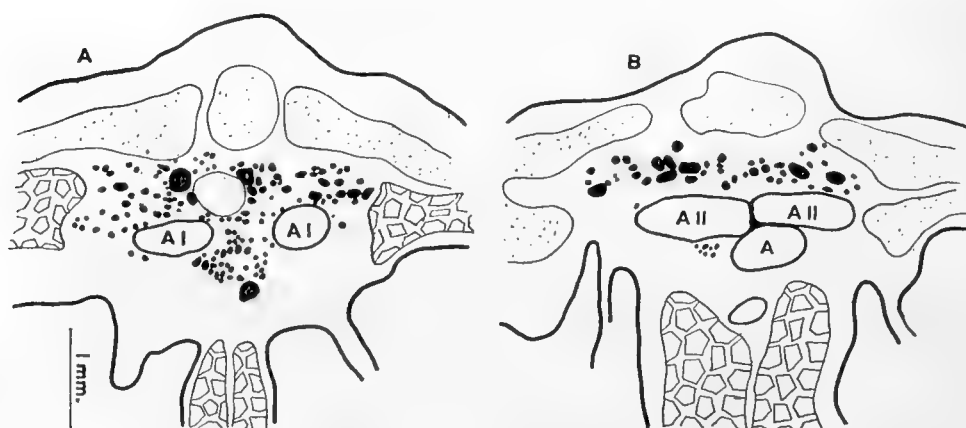


Fig. 15. Sections through the thyroid gland of *Stenotomus*. *A*, in the aortic bifurcation; *B*, at the second branchial arteries.

STENOTOMUS CHRYSOPS L.

*Length of specimen 25 cm.* The scup presents the thyreoid gland as a rather continuous organ, only one group of follicles lying below the aorta is isolated from the main mass. The largest expansion of the gland is in (fig. 15, A) and immediately behind the aortic bifurcation; here it measures 3 mm. in width, and dorso-ventrally over 1 mm. This expansion is followed by a restriction, the follicles always lying above the aorta. At the second branchial arches another increase in the thyreoid tissue occurs, and here a few follicles appear below the aorta (pl. I, fig. 14).

The size of the generally circular follicles varies from 20 to 300 $\mu$  in diameter, a few reaching 400 $\mu$ .

CYNOSCION REGALIS BLOCH

*Specimens of 60 cm. in average length.* Twelve specimens of the squeteague were examined and they serve to show a series of variations in the thyreoid gland within the species. The region of the gland extends from in front of the aortic bifurcation to the third branchial arteries. The majority of follicles always lie either dorsal or lateral to the aortic stem and in only two cases were any follicles found below the aorta. In one case the aortic stem between the first and second branches was surrounded. The region of the second aortic branches is commonly filled by the gland. The tendency to extend from this place anteriorly is more often expressed than in the opposite direction. The lateral extension is greater along the branchial arteries than in intermediate regions (pl. III, figs. 31-41).

In some of the specimens there were two (pl. III, figs. 33, 36, 37, 40) or even three and four (pl. III, fig. 34) well developed isolated portions of the gland lying on different branches of the gill vessels. Macroscopically they appear to be separated, but on tracing the entire region in serial sections it is found that follicles spread out and connect the several masses, although the follicles are small and scattered so thinly that they were not seen with the naked eye.



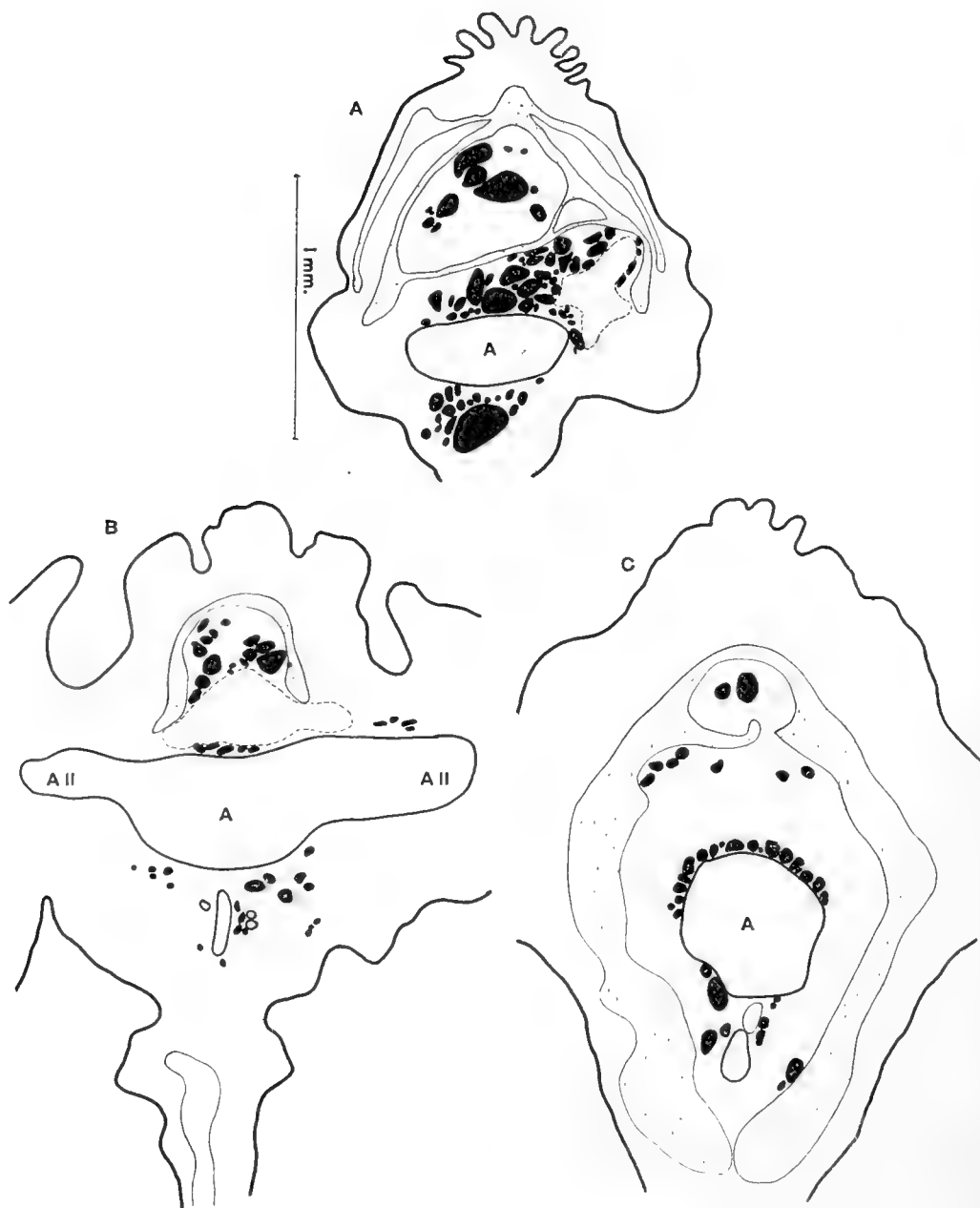


Fig. 16. Sections through the thyroid gland of *Micropogon*. A, between the first and second; B, at the second; C, near the third branchial arteries.

The mass of thyroid tissue, roughly judging, differed in the specimens, although they were of about the same size, yet the fish may have been of different ages.

The shape of the follicles varies from very irregular to circular. Their size also varies extremely. Those lying nearest the vessels are huge and irregular, while the small peripheral ones approach

a globular shape. This indicates that the shape of the follicles is a result of the pressure directions. The follicular arrangement is rather compact in the central portions. (pl. V, fig. 17.)

The epithelial cells vary from low cuboidal to high cylindrical shapes. The smaller follicles seem to have a little higher epithelium, though it is rather uniform in the same individual and varies more among the several specimens. It may seem therefore that the entire gland is in the same physiological stage.

#### MICROPOGON UNDULATUS L.

*A croaker, 30 cm. long.* The thyreoid extends from the first to the third branchial arteries (pl. II, fig. 15). The dispersion of follicles is largely dorso-ventral, since laterally they are hindered by the narrowness of the isthmus (fig. 16, A, C). For this reason also a considerable part of the gland lies below the aorta, yet not so large a portion as above, though the dorsal follicles are less densely arranged.

There are only a few follicles in front of the aortic bifurcation, yet at the bifurcation and behind it lies the main mass of the gland. The follicles completely fill the spaces between bones and vessels (fig. 16, A). Towards the second gill branches the copula extends further and further down and forces the follicles into a somewhat lateral position. The ventral mass is larger in this region. At the second arterial branches there is no special increase in mass, the number of ventral follicles having decreased (fig. 16, B), the dorsal ones increasing and soon extending to the epithelium of the pharyngeal floor. The follicles lie rather loosely arranged, but have not noticeably increased in size. A small line of follicles above the aorta extends from here towards the third gill branches, others are scattered irregularly around the aorta. The aorta has sunk into the ventral muscle and carries the posterior follicles with it.

The thyreoid gland of *Micropogon* is characterized by rather small follicles of almost uniform size, though in some regions large ones appear. The diameters range from 10 to 300 $\mu$ , but those of 30 to 50 $\mu$  are most abundant.

The epithelium is rather low, even in the smallest follicles. Branched follicles are numerous. The blood supply is rich, many capillaries being present around the follicles. There are several larger veins running through the thyroid region.

#### TAUTOGOLABRUS ADSPERSUS WALBAUM

*Length of specimen 25 cm.* In the cunner the thyroid gland occupies a unique position, almost resembling that in the sharks. It is pushed far forward in the aortic bifurcation, and touches both the first branchial arteries laterally (fig. 17, *B*), but does not extend far enough back to come into contact with the ventral aorta (pl. II, fig. 16). The main mass is, as it were, imbedded in a bony capsule. The follicles are grouped around a median vein (fig. 17, *A*). Dorsal to the aortic bifurcation the copula and a transverse muscle are well developed, so that the thyroid is forced forward. The follicles are not numerous, and are all more or less irregular. Their diameters measure from 15 to 200 $\mu$ . The follicular epithelium is cuboidal.

#### TAUTOGA ONITIS L.

*Specimen 35 cm. long.* In the closely related tautog the thyroid gland also occupies a rather cephalad position (pl. II, fig. 17). It extends back from within the aortic bifurcation almost to the second branchial arteries. It lies chiefly above and to the sides of the aorta. The anterior, main part, is imbedded in an osseous capsule which is square in cross section, and is formed by three branchial bones above and a ventral supporting bone (fig. 18, *A*). At the aortic bifurcation the capsule becomes incomplete and the follicles are widely dispersed over 6 mm. (fig. 18, *B*). The follicles follow the dorsal side of the first arterial branches out to the base of the gills. Behind the first branchial arteries the lateral extension decreases and the follicular dispersion is in a dorso-ventral direction. The follicles are loosely arranged, and yet globular ones are rare, most of them being polygonal in outline. The average size is 150 $\mu$  in diameter, but there are a few giant folli-

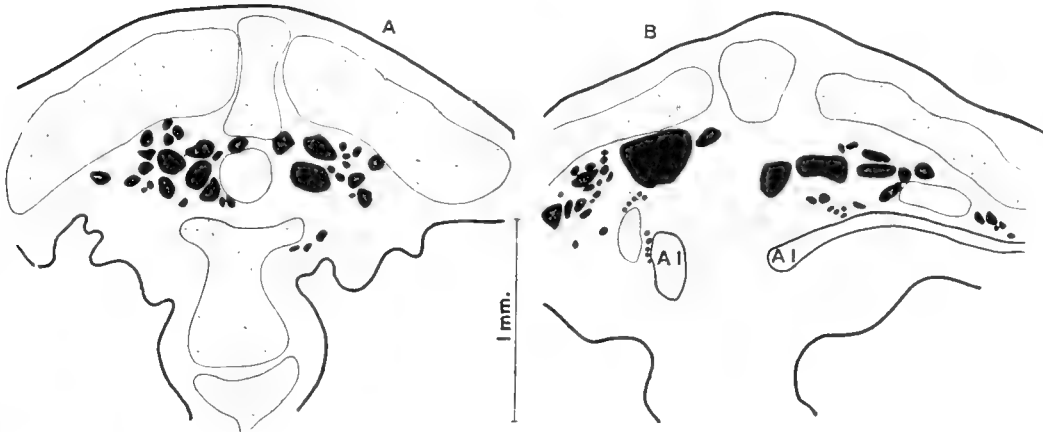


Fig. 17. Sections through the thyroid gland of *Tautogolabrus*. *A* and *B*, anterior to the aortic bifurcation.



Fig. 18. Sections through the thyroid gland of *Tautoga*. *A*, anterior to; *B*, at and *C*, posterior to the aortic bifurcation.

cles,  $700\mu$  long by  $400\mu$  broad. Branched follicles are numerous. The epithelial cells are cuboidal in shape.

#### PRIONOTUS CAROLINUS BLOCH

*In a sea-robin 30 cm. long* the thyroid gland seemed to show a pathological appearance. The invasion of the surrounding tissues by thyroid follicles was extraordinary, but may be abnormal. For this reason it can only be stated that the gland in this species occupies a posterior position, close to the origin of the truncus arteriosus.

#### OPSANUS TAU L.

*A toadfish 30 cm. long* the gill region in this species is extremely shortened, and therefore the thyroid region begins rather far forwards. Anteriorly the largest follicles lie on both sides of a process of the copula which extends ventrally (fig. 19, A). Towards the aortic bifurcation the size of the follicles decreases and the two lateral portions unite in the median line, at the same time the lateral extension (fig. 19, B) of the follicles increases remarkably (pl. II, fig. 18). Some follicles appear below the aortic stem. Between the first and second branchial arches the number of follicles decreases above the aorta, while ventrally they disappear entirely. Along the second branchial arteries the follicles again reach laterally and also again appear ventrally. Behind this point the aorta sinks more and more and the space around it becomes freer. Yet there is no special increase of thyroid tissue in this region, there being only loosely scattered small follicles. A few follicles accompany the aorta in its course into the space between the musculus sternohyoideus. The caudal end of the thyroid lies behind the third gill branches.

The arrangement of the follicles is loose, and they are usually circular in cross sections. Some are flattened between the bony and muscular surrounding tissues. Their size varies extremely. The largest ones,  $600\mu$  in diameter, lie in the anterior end, which is the reverse of the general rule for other species. In other regions

the follicular diameters vary from  $50\text{--}400\mu$ , the median size follicles being the most abundant.

The follicular epithelium is always cuboidal. Colloid is present in almost all the follicles, and is very brittle and homogeneous. In the larger follicles the colloid stains much lighter than in the

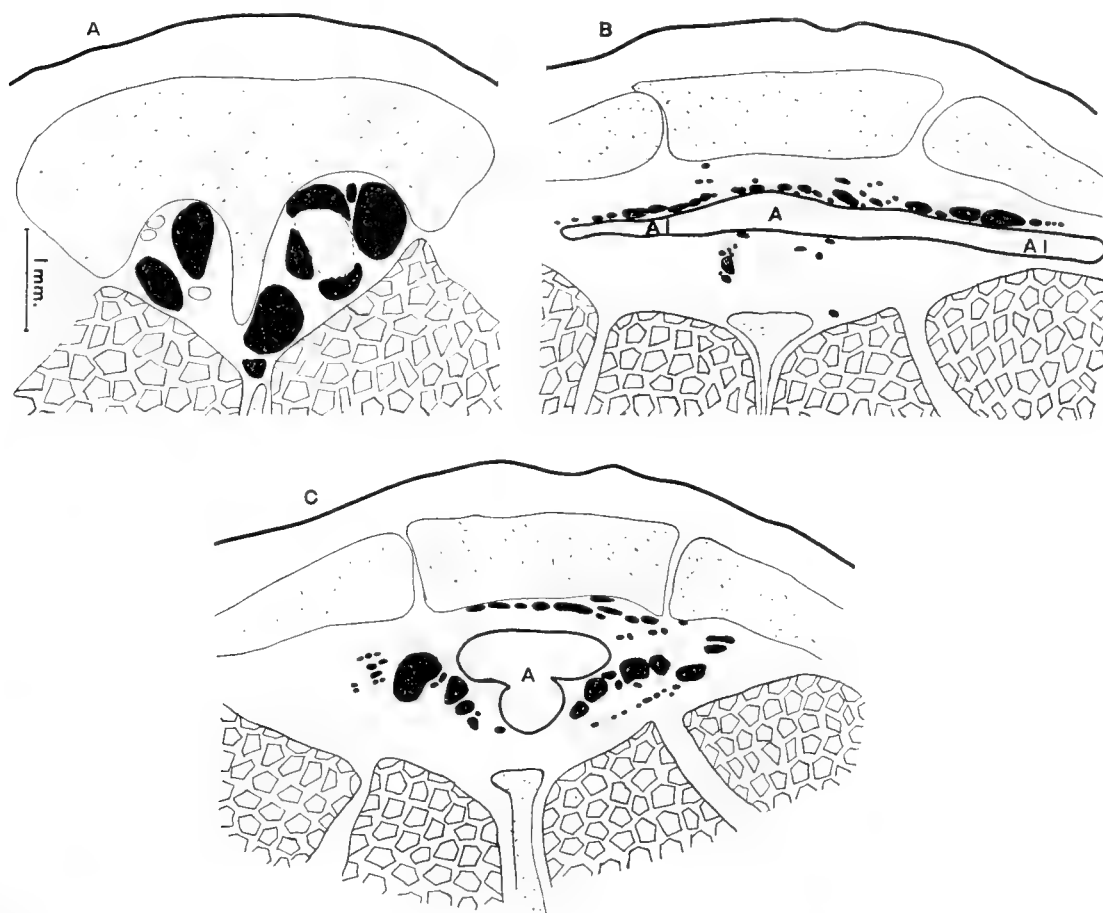


Fig. 19. Sections through the thyroid gland of *Opsanus*. A, anterior to; B, at the aortic bifurcation; C, at the second branchial arteries.

smaller. Lymphocytes are numerous within the follicles. The blood supply of the thyroid region is very poor.

#### MURAENOIDES GUNELLUS L.

*Length of specimen 40 cm.* The thyroid gland in the butterfish reaches a considerable size (pl. II, fig. 19). The anterior end lies in front of the aortic bifurcation and consists only of small



Fig. 20. Sections through the thyroid gland of *Muraenoides*. *A*, anterior to the aortic bifurcation; *B*, between the first and second; *C*, close to the second branchial arteries.

scattered follicles. In the middle portion of the gland the follicles are numerous and closely arranged. At the aortic bifurcation they lie around a large vein and completely fill out the space between the gill arch and muscles. The lateral extension of the follicles is small as compared with the dorso-ventral, since the isthmus is narrow (fig. 20, *B*). A cross section through the thyroid area measures about 1 mm. Taking 1 mm. as the average width we would have 10 cubic mm. of thyroid tissue in this species.

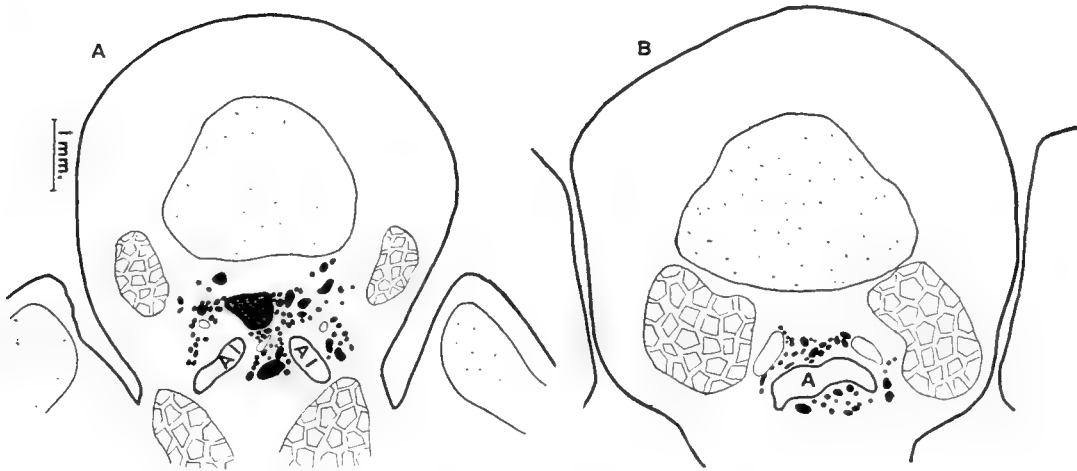


Fig. 21. Sections through the thyroid gland of *Pleuronectes*. *A*, in the aortic bifurcation; *B*, just posterior to it.

The first branchial arteries are partly surrounded by follicles. Behind the aortic bifurcation there is an open space for lateral extension, but not for ventral, since the aorta rests on the musculature. The caudal end of the gland lies a little behind the second branchial arteries, and consists again of small scattered follicles (fig. 20, *C*).

The follicles are of globular or long ovoid shape, some are very irregular. The circular cross sections vary from 20 to 500 $\mu$  in diameter. The very large ones lie at the second arterial branches. Branched follicles with connecting channels between them are numerous, so that almost all follicles may be traced in sections as evaginations of others.

The follicular epithelial cells vary from highly cylindrical, narrow shapes to broad cuboidal. Flattened epithelium is rare. The cells are extremely numerous and densely arranged. The nuclei are located near the base of the cells, even in the higher ones, and the cytoplasm stains darker about the nucleus. Sometimes it appears as if there were a cuticle on top of the cells, as many authors have described. This, however, is nothing else than a refractive appearance of the cell margin from which the cytoplasm has slightly retracted. The blood supply is extremely rich.

(A parasitic worm was found in this thyroid and had caused a considerable hemorrhage.)



## PSEUDOPLEURONECTES AMERICANUS WALBAUM

*Length of specimen 45 cm.* The position of the thyroid gland in the flounder varies (pl. III, figs. 30, 31). In one case it formed a rather compact nodule between the first and second branchial arteries, while in another the main mass was found in the aortic bifurcation between and surrounding the first branchial branches (fig. 20, A). Behind the aortic bifurcation there were only smaller follicles dorsal and lateral to the aorta. The broad base of the deep reaching copula permits only a lateral extension; thus the thyroid presents itself as a transverse streak. Small detached follicles lie close to the base of the gills.

The size of the follicles varies from 15 to 1000 $\mu$  in diameter, those of about 200 $\mu$  being in the majority. There are also a few 'giant' follicles. The epithelial cells of the follicles are closely arranged and rather high. The nuclei are oval. The blood supply is rich and lymphatic vessels are well developed.

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## PLATES

#### EXPLANATION OF PLATES I TO III

Plates I and II show the regions of distribution of the thyreoid elements in different species. I, aortic bifurcation or first branchial arteries; II, III and IV, the second, third and fourth branchial arteries.

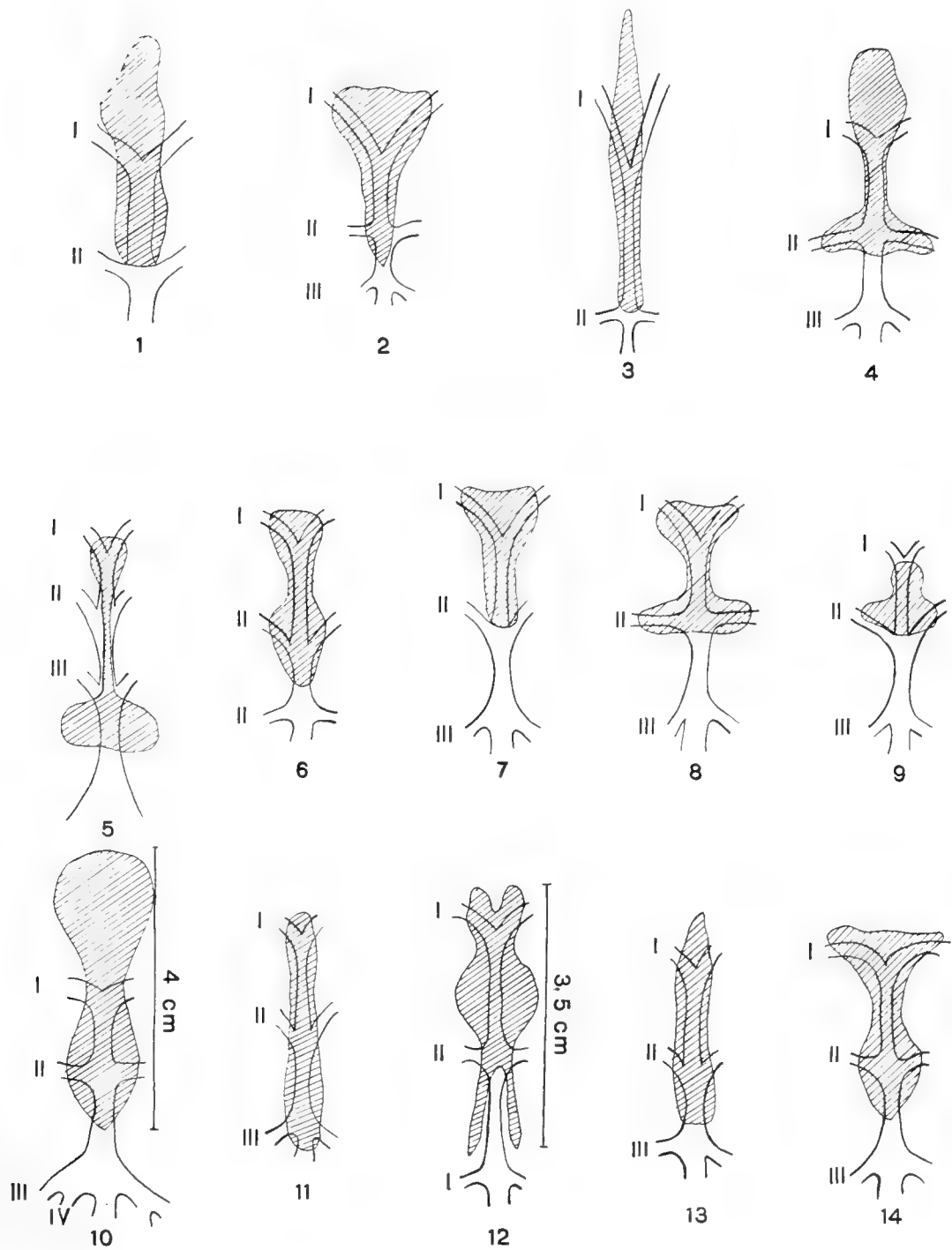
- |                                |                                       |
|--------------------------------|---------------------------------------|
| 1 <i>Anguilla chrysypa</i>     | 14 <i>Stenotomus chrysops</i>         |
| 2 <i>Clupea harengus</i>       | 15 <i>Micropogon undulatus</i>        |
| 3 <i>Brevoortia tyrannus</i>   | 16 <i>Tautogolabrus adspersus</i>     |
| 4 <i>Osmerus mordax</i>        | 17 <i>Tautoga onitis</i>              |
| 5 <i>Siphostoma fuscum</i>     | 18 <i>Opsanus tau</i>                 |
| 6 <i>Fundulus heteroclitus</i> | 19 <i>Muraenoides gunellus</i>        |
| 7 <i>Fundulus diaphanus</i>    | 20 <i>Oncorhynchus kisutch</i>        |
| 8 <i>Fundulus majalis</i>      | 21 <i>Salmo mykiss</i>                |
| 9 <i>Menidia notata</i>        | 22 <i>Salmo irideus</i> , age 1 month |
| 10 <i>Sarda sarda</i>          | 23 <i>Salmo irideus</i> , age 1 year  |
| 11 <i>Pomatomus saltatrix</i>  | 24 <i>Cristivomer namaycush</i>       |
| 12 <i>Morone americana</i>     | 25-27 <i>Salvelinus fontinalis</i>    |
| 13 <i>Mugil cephalus</i>       |                                       |

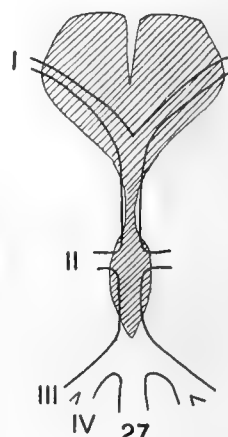
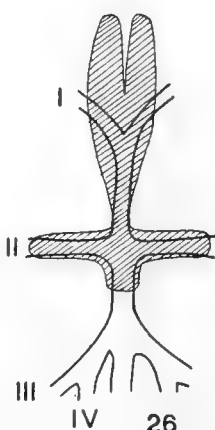
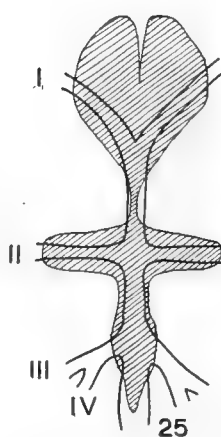
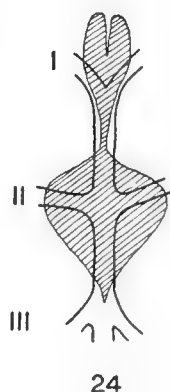
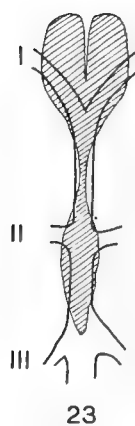
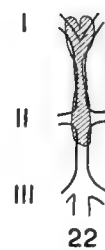
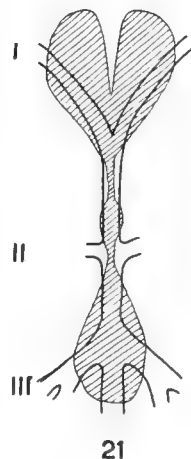
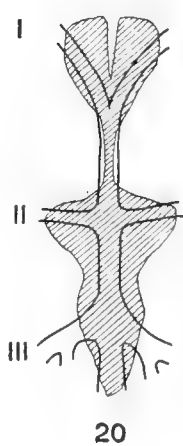
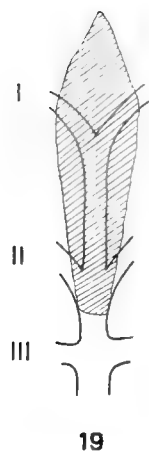
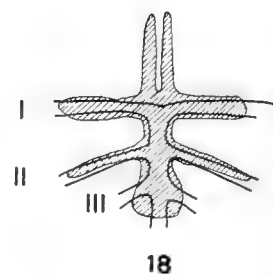
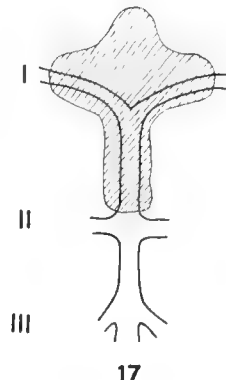
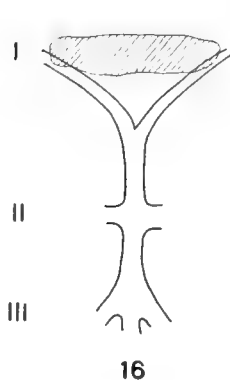
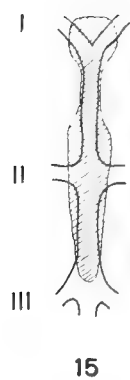
Plate III, Diagrams of actual portions of the thyreoid gland visible to the naked eye.

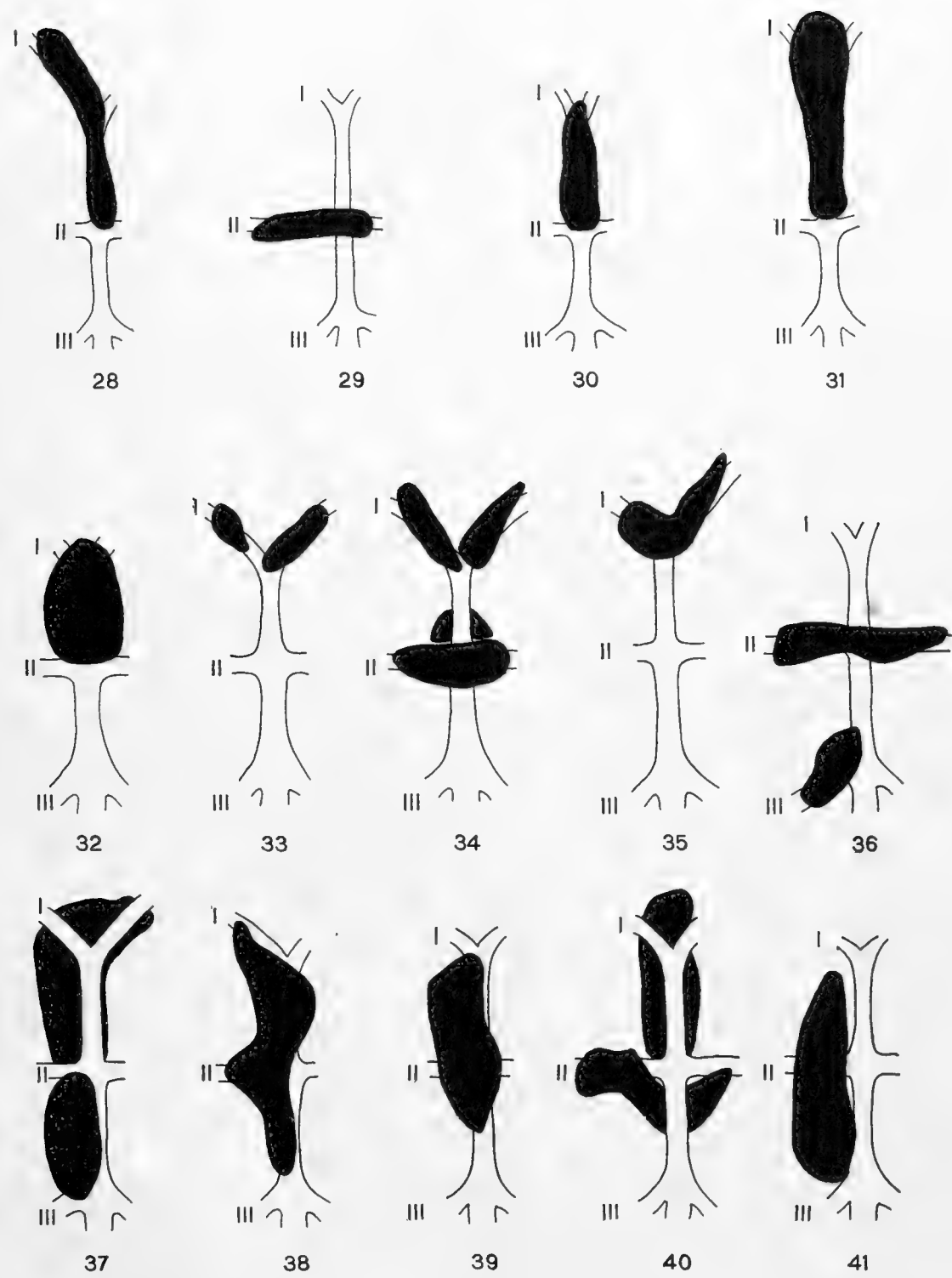
28 and 29 *Salvelinus fontinalis*

30 and 31 *Pseudopleuronectes americanus*

32-41. Ten specimens of *Cynoscion regalis*, demonstrating the great variability in extent and position of the organ within the species.









#### EXPLANATION OF PLATE IV

Plates IV and V, Photographs of the histological features of the thyreoid gland in different species.

1 Brevoortia. Two follicles with their epithelial cells drawn out into spinous processes. Dia. 1:165.

2 Brevoortia. Two follicles, *F* (the right one containing colloid), and a large lymph vessel, *L*, between them. The content of the vessel shows similar droplets to those sometimes seen on the surface of colloid, and believed by Anderson to contain the 'chromophobe' secretion. Dia. 1:350.

3 Brevoortia. An isolated follicle, *F*, in the most anterior portion of the thyreoid, with neighboring lymph vessels, *L*. Dia. 1:160.

4 Brevoortia. General view of the thyreoid in the osseous capsule. Dia. 1:60.

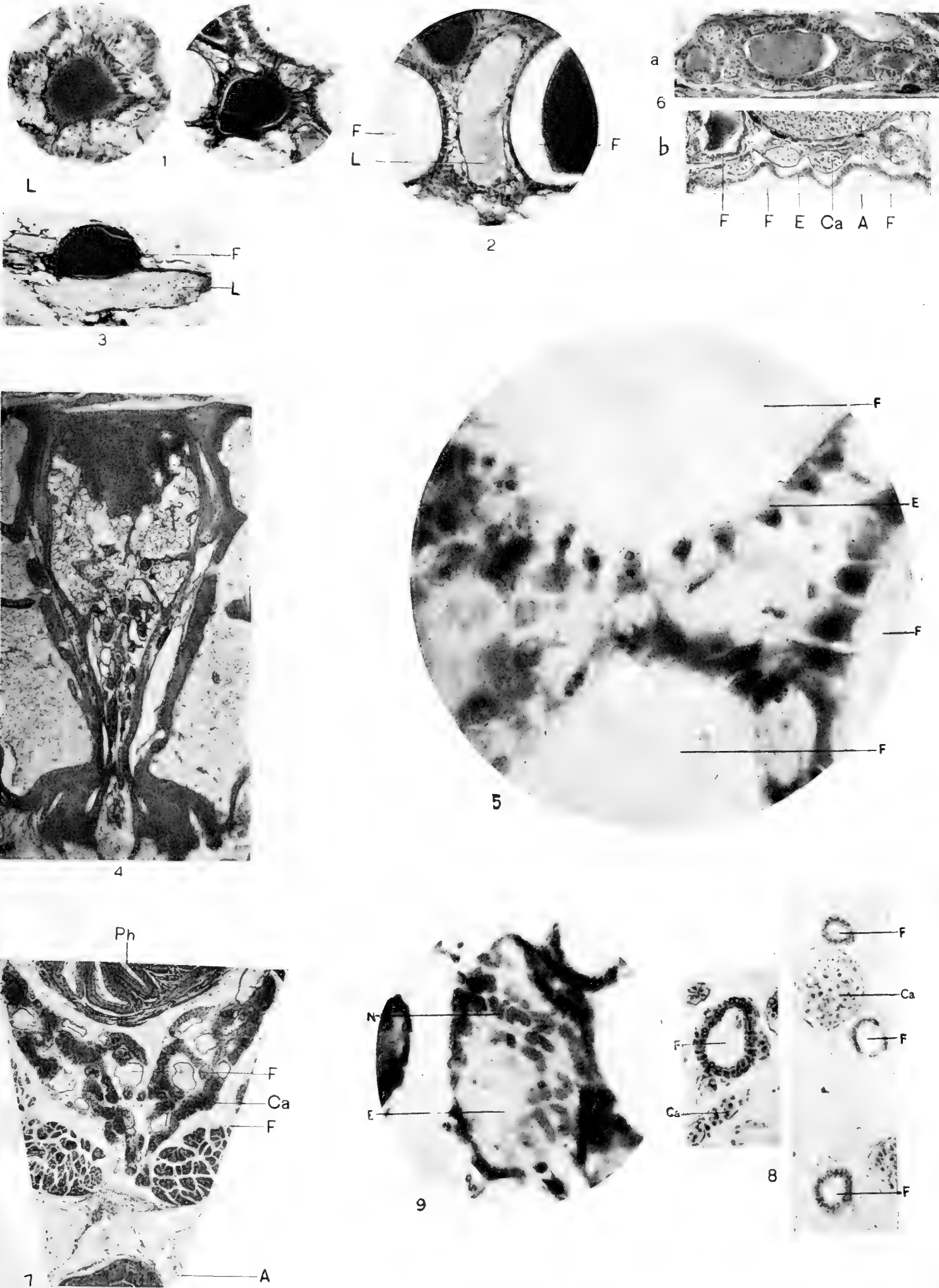
5 Brevoortia. Degenerating epithelial cells and their basal processes. *F*, Follicular lumen, *E*, Epithelium. Dia. 1:700.

6 Fundulus heteroclitus. Two pictures showing numerous small capillaries, *Ca*, deeply buried in the epithelium, *E*, of the follicles, *F*. In the lower picture the epithelium has retracted slightly from the endothelium. *A*, ventral aorta. Dia. in a, 1:134; in b, 1:345.

7 Siphostoma. A general view of the posterior end of the thyreoid gland. *Ph*, pharynx; *A*, ventral aorta; *F*, follicles; *Ca*, capillaries. Dia. 1:34.

8 Siphostoma. Single follicles, *F*, with their colloid forming epithelial cells, but containing no colloid. *Ca*, capillaries. Dia. 1:345.

9 Oncorhynchus. Irregular nuclei, *N*, of the epithelial cells. *E*, epithelium of a follicle viewed from the top. Dia. 1:650.



Jaches Photo.

#### EXPLANATION OF PLATE V

10 *Salvelinus fontinalis*, spec. no. III. Capillary network, *Ca*, around the follicles. Note the highly columnar epithelium of the upper and the cuboidal epithelium of the two lower follicles. Dia. 1: 134.

11 *Salvelinus fontinalis*, spec. no. II, showing different heights of the epithelial cells in the same follicle. Dia. 1: 375.

12 *Salvelinus fontinalis*, spec. no. II. Distribution of the thyroid elements and their capillaries, *Ca*, in the connective and fatty tissue network. Dia. 1: 64.

13 *Salvelinus fontinalis*, spec. no. III. Much swollen colloid forming cells, *Coz*, which have been cast off from the follicular wall into the colloid, *Co*. *E*, epithelium; *N*, nucleus; *V*, vesicles in the colloidal substance. Dia. 1: 650.

14 *Oncorhynchus*. A general view of the thyroid gland between the first and second branchial arteries. *Ph*, epithelial floor of the pharynx; *F*, follicles surrounding the copula; *A*, ventral Aorta. Dia. 1: 60.

15 *Anguilla*. A duct, *D*, connecting a small, *f*, and a large thyroid follicle, *F*. Dia. 1: 165.

16 *Anguilla*. This photograph shows a complex of follicles which, on tracing through the series, are found to connect with the follicle, *F*, on the right side of the illustration. Dia. 1: 170.

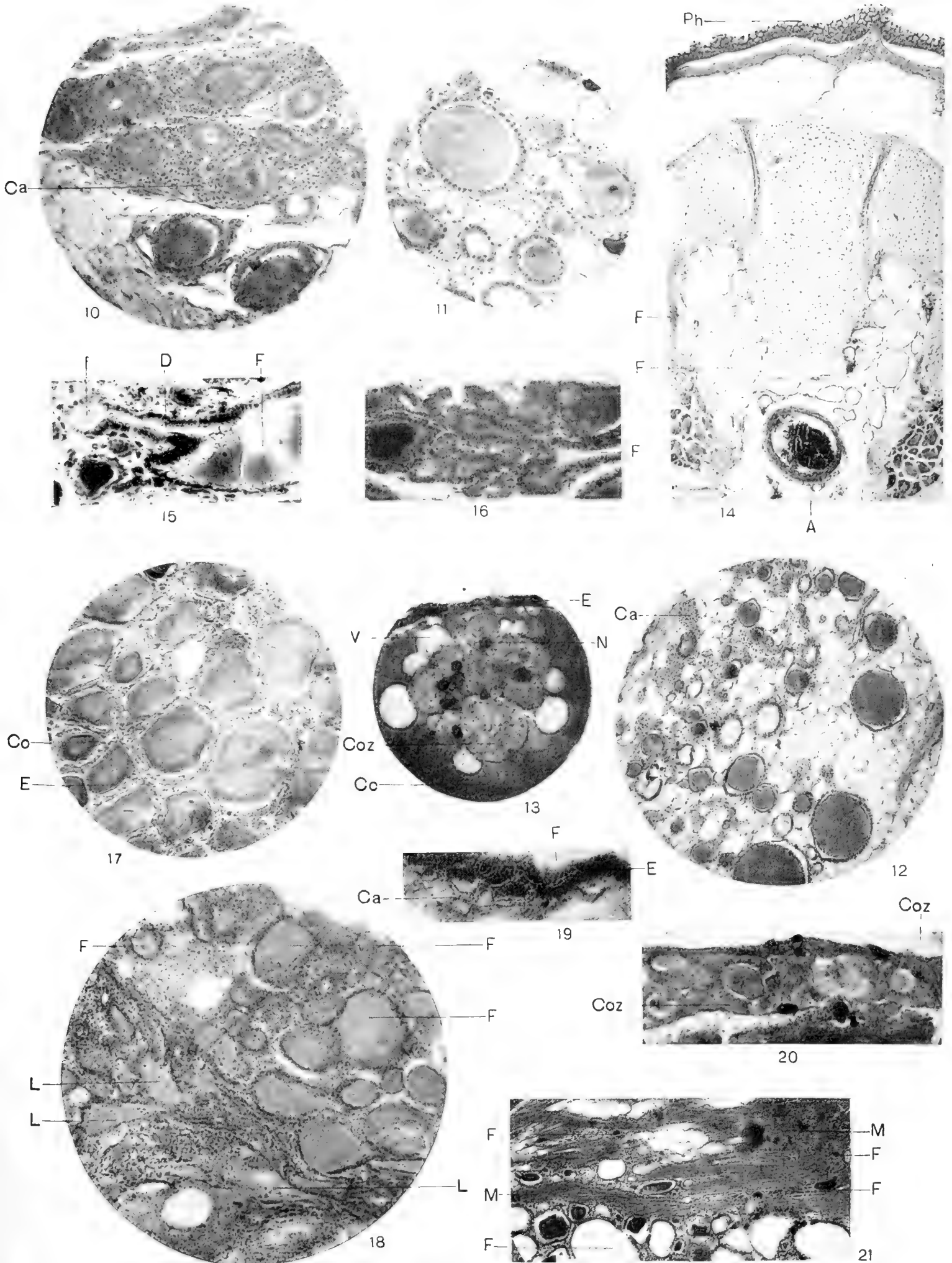
17 *Cynoscion*. A general view of the densely arranged follicles of this species. *Co*, colloid; *E*, epithelium. Dia. 1: 165.

18 *Cynoscion*. This picture shows the ramifying lymph spaces, *L*, completely filled with the same substance as the follicles, *F*. Dia. 1: 170.

19 *Tautoga*. The epithelial wall, *E*, of the follicle, *F*, is cut somewhat tangentially so that the network of anastomosing capillaries, *Va*, enclosing the follicles can be seen. Dia. 1: 145.

20 *Clupea*. Much swollen colloid forming epithelial cells, *Coz*, in a colloid zone. Dia. 1: 170.

21 *Sarda*. Showing smooth muscle bundles, *M*, invaded by thyroid follicles. Dia. 1: 60.



Jaches photo.













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